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COMPARATIVE SYMPTOMATOLOGY OF PSOROSIS VARIETIES ON CITRUS IN CALIFORNIA[1]

H. S. Fawcett[2] and A. A. Bitancourt[3]

(Accepted for publication March 3, 1943)

Studies and observations during the last few years have shown that a number of symptoms on citrus, previously considered those of totally different diseases, can now be connected with the virus disease psorosis through certain features of these symptoms on both bark and leaves (5, 6, 10, 11). The use of the term *psorosis*[4] is, therefore, expanded to cover a group of disorders now thought due to varieties of one virus. These disorders include psorosis A, psorosis B, concave gum, blind pocket, crinkly leaf, and others of minor importance, to be mentioned later. The last three are, accordingly, now designated as varieties of psorosis and called "concave-gum psorosis," "blind-pocket psorosis," and "crinkly-leaf psorosis." Since transmission of these varieties of psorosis has been possible only through use of living cell tissue, the present grouping is made on the basis of certain characters and symptoms that are common to all.

The young-leaf symptoms, although somewhat variable within each variety of psorosis, are those most consistently similar throughout the entire group. Another feature, fundamentally similar in the different varieties of psorosis when viewed in broad aspect, is an alteration near the cambium in the primary lesion of the wood, the varieties differing chiefly in the extent of the alteration produced.

It is the object of this paper to describe the gross external and internal characters of the different varieties of psorosis in some detail and to point out the common features as well as the differences. Some other, minor effects, occurring on citrus in California, possibly due to a related virus or viruses, are described briefly.

YOUNG-LEAF SYMPTOMS OF ALL THE PSOROSIS VARIETIES

Since the young-leaf symptom (3, 4) is common to all the psorosis varieties, and since its similarity in the varieties seems to tie them together into one group, this character will be described before differentiating the varieties.

Small, elongated areas, white to yellowish or distinctly lighter to slightly paler green than the rest of the leaf blade, occur on the leaves in the region of the veinlets. These white to light-green areas are usually about 1 to 3

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[4] *Psora* = a cutaneous disease, the itch, scabies; *osis* = state or condition. The name was first suggested by Swingle and Webber (14).

mm. long and $\frac{1}{4}$ to 1 mm. broad, the long axis being parallel with the main side veins (Fig. 1). They may be very numerous and scattered over the entire blade, or they may occur only on certain limited portions of the leaf blade. Sometimes only a small percentage of leaves of suitable growth

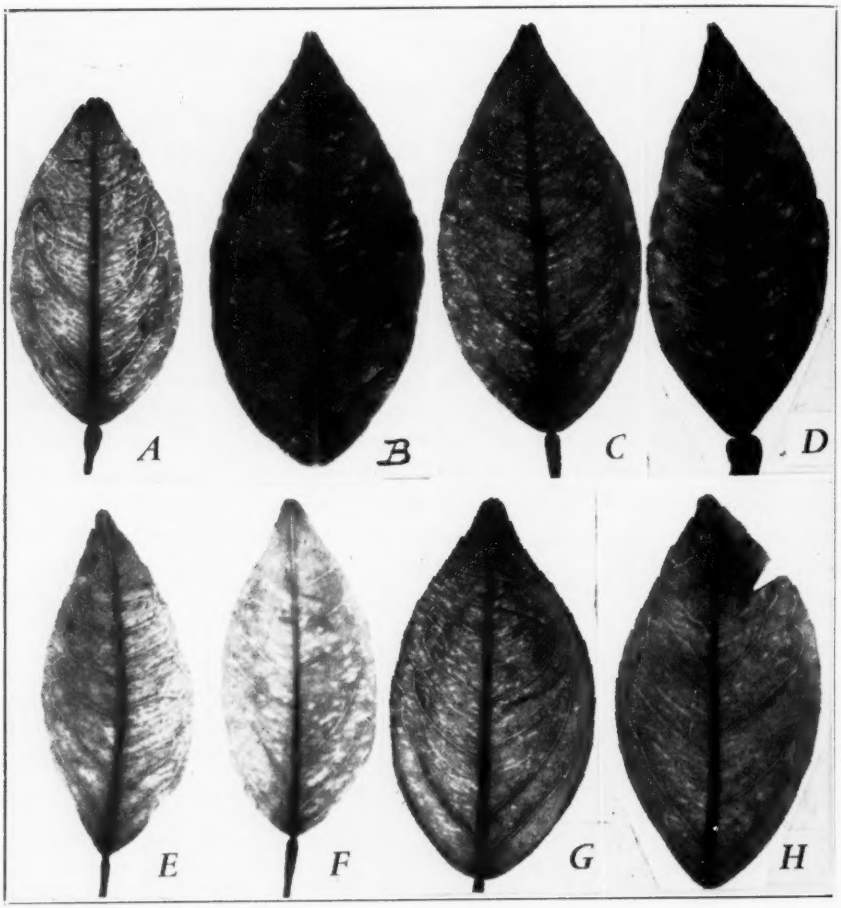

FIG. 1. Some of the most common aspects of young-leaf symptoms of psorosis, on orange leaves. Even greater variations in size and distribution of the cleared areas than here shown can be found in connection with each of the varieties of psorosis. The young-leaf symptoms can therefore not be used to distinguish the varieties. These leaves were from trees affected as follows: A and B. Psorosis A. C and D. Psorosis B. E and F. Blind-pocket psorosis. G. Crinkly-leaf psorosis. H. Concave-gum psorosis. All × about $\frac{3}{4}$.

shows these symptoms, but at other times a great majority of the leaves will show them. On young, tender leaves, one-quarter to one-half grown, the veinlets, as well as tissue adjacent to or between them, may show faint to pronounced clearing. It is not uncommon for a leaf or two to show distinct symptoms, while other leaves of apparently similar age on the same twig or on adjacent twigs show no visible symptoms.

Often the small flecks are indistinct and fade out gradually at the margin or coalesce and form a large light-green area. Sometimes a clearing extending along the veins in the form of a band is the principal feature.

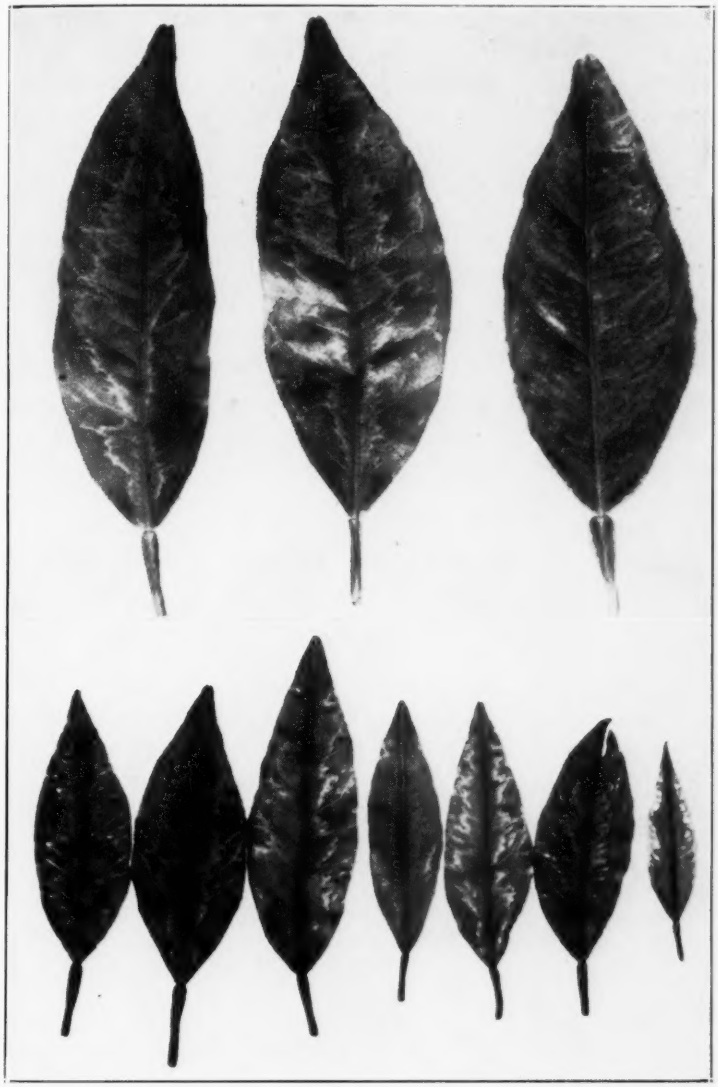

FIG. 2. The zonate or oak-leaf pattern of psorosis on young orange leaves, seen especially in the spring and most commonly on trees affected with concave-gum psorosis. Leaves in upper row about natural size; those in lower row × about ¾. Photographed by L. J. Klotz.

Frequently, the cleared region forms a distinct, characteristic pattern known as the zonate or oak-leaf pattern (Fig. 2). This is seen in the spring or early summer and is most commonly associated with the concave-gum pso-

rosis. At other times of the year, this pattern is not present even on trees affected with concave gum, and the young-leaf symptoms are then indistinguishable from those of the other psorosis varieties.

To see these symptoms most readily, the young quarter- to half-grown leaves should be shaded from the direct sun and viewed with strong transmitted light or with the light of the sky coming through the blade. The light-yellow to pale-green areas or flecks will show in contrast to the darker green of the rest of the blade (3, 4, 5, 6).

Caution is necessary to distinguish between psorosis symptoms and leaf injuries caused by thrips, red spider, small sharp hail stones, minute sand grains driven by wind, or the slight rubbing of the leaves against branches, all of which may produce markings similar in size to those of psorosis. If the leaf is turned over slowly in the direct sunlight, such injuries can often be detected and discounted.

As the leaves mature, the cleared areas or young-leaf symptoms caused by the psorosis disappear. On the mature leaves, other symptoms, such as circular spots, ring spots, concentric-ring spots, gum excrescence, blotches, crinkle, variegation, etc., may develop; but since these symptoms are not common to all the psorosis varieties, they are not described until later.

PSOROSIS A

Psorosis A, which is caused by the virus *Citrivir psorosis* var. *vulgare*[5] (7, 8), is by far the most common variety of psorosis. Although slower to develop deleterious effects and less serious in its effect on a given individual tree, psorosis A is responsible for much more damage than is psorosis B.

The citrus species most severely affected by psorosis A, in respect to bark symptoms, are sweet orange, grapefruit, and tangerines, but other species show the leaf symptoms. In severity, psorosis A ranges from very mild to very severe. Some strains of the virus produce bark symptoms on sweet-orange trees within 6 years from the time of budding, while others may take 15 to 20 years. It is possible that some strains produce only leaf symptoms and never bark symptoms during the usual life of the tree. One form is found occasionally on the bark of sour orange, a variety usually resistant to bark lesions. How much of this variation is due to difference in virulence of strains and how much to differences in host resistance under different conditions, is not yet clear.

Bark Symptoms

The symptom expression on the bark usually begins either as small scales or flakes of outer bark (Fig. 3, B and C), with or without gum formation, or as aggregations of small, erumpent pustules (Fig. 3, A), under which the tissue is brown. The scales of outer bark are dry, irregular flakes, about $\frac{1}{12}$ to $\frac{1}{8}$ inch in thickness, which separate and peel away, exposing the tan- to

[5] *Citrivir*, from *citri* (Latin genitive of *citrus*) and *vir* (stem of *virus*) = virus of citrus; *psorosis* (Latin genitive of *psorosis*) = of the disease known as psorosis; *vulgare* = common.

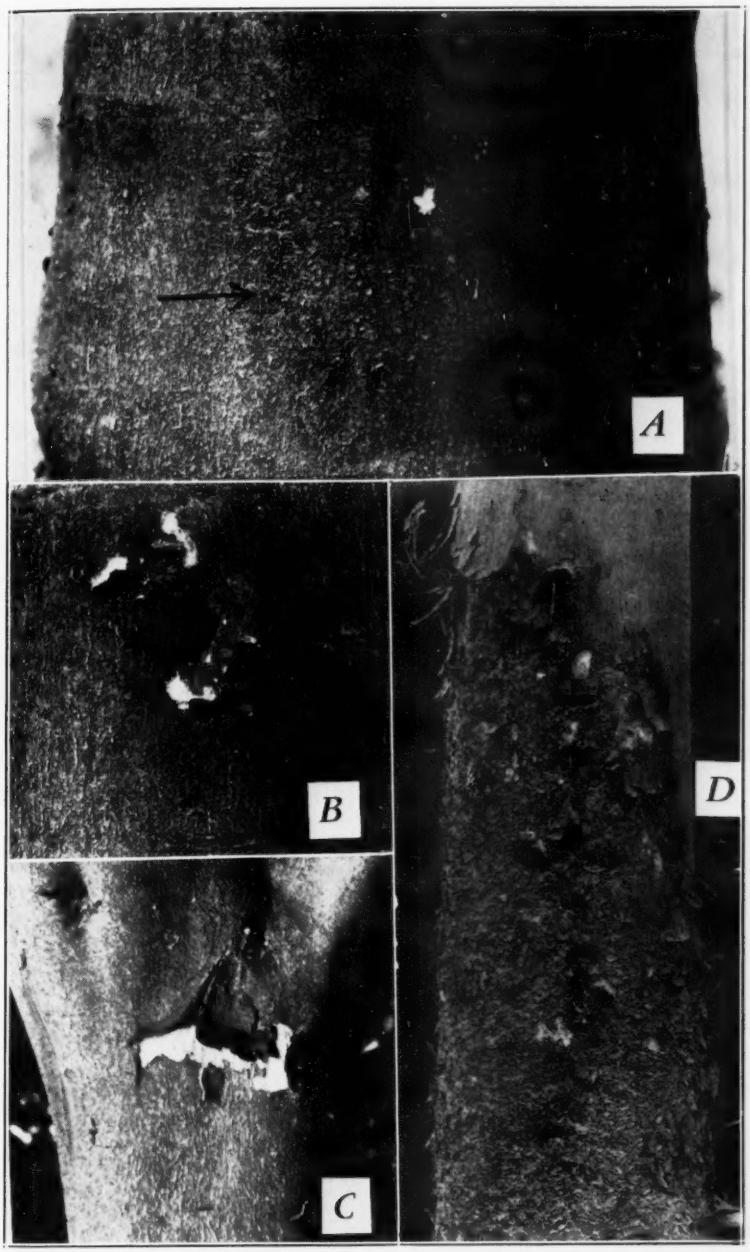

FIG. 3. Psorosis A. Different stages and forms of bark symptoms on trunks of orange trees. A. Early stage: aggregations of small, erumpent pustules—the pimply form in central portion. × about $\frac{3}{4}$. B. and C. Early stages of scaling. B, × about $\frac{3}{4}$; C, × about $\frac{1}{2}$. D. Later stage of scaling. × about $\frac{1}{2}$.

buff-colored surface of the live layer of bark underneath. These scales or pustules, as the case may be, usually occur first in localized areas on the bark of the trunk or limbs of trees 6 to 12 or more years of age (rarely earlier). As the scaling advances (Fig. 3, D), the deeper layers of bark are affected by irregular growth and by gum or gumlike deposits. Small or large amounts of gum often form, the amount depending upon weather and growth conditions and probably upon the strain of the virus (5). The rate of scaling varies greatly on different trees and probably represents the differential effects of strains.

The histological aspects of psorosis A in affected bark have been given in some detail by Webber and Fawcett (15) and by Fawcett (5). In brief, yellow-to-brown contents are first seen in some of the parenchyma cells of the phelloderm and of the primary cortex of the outer bark underlying the affected surface. Later, some of the groups of brown cells near the surface are cut off by a phellogen or cork-cambium layer producing phellem or cork cells toward the outside and phelloderm on the inside. These outside layers, cut off by the cork layer, die and form the dry scales or flakes of bark.

The increased phelloderm in diseased bark causes excessive thickening and frequently contains, in addition to the parenchyma, broken bands of enlarged stone cells. After the primary cortex has scaled off, the browning continues in the newly formed phelloderm and later extends into the living phloem. With further progress, the irregular contour of the cork cambium, which continues to produce an abnormal amount of phelloderm, creates pressures resulting in distortions within the underlying tissues. Microchemical tests of the brown contents in certain parenchyma cells, mentioned above, indicate that they may contain phenolic compounds, fatty substances, gum, and even ligninlike substances. Gum pockets involving only parenchyma tissue occur in thick phelloderm. In badly diseased tissue, the entire cortex and most of the phloem may be replaced by a group of wood cells surrounded by cambium.

Wood Symptoms

Primary Lesions. Coincident with or following the development of the bark lesions, gum deposits are formed within and between layers of wood, more or less corresponding to seasonal and annular rings of growth. These layers of gum, since they comprise the first visible effects in the xylem region, are described as the primary lesions of the wood (Fig. 4). The continuous regions of gum are apparently formed by the dissolution of embryonic cells, usually involving only cells between rays. Cells bordering the gum regions may be filled with gum. Some cells of wood tissue produced at periods of growth when conditions do not favor the formation of gum regions, are normal or may contain lignified, thin-walled isodiametric cells with pits showing indistinct borders. Plugging of vessels, due to the accumulation of gum in the vicinity of perforated plates, is often seen in apparently normal wood between the gum layers before the secondary

lesions develop. The rate of wood growth becomes irregular in some psorosis lesions (15).

Secondary Lesions. A number of years after the inception of the bark lesions and the formation of the primary lesions of the wood containing gum regions, important changes take place in the deeper wood layers. These alterations do not usually begin until the lesions have become extensive, usually not before the trunk or limb is girdled by the bark lesions, with their accompanying primary lesions in the peripheral wood layers beneath.

In the beginning stage, these alterations, or secondary lesions, are seen

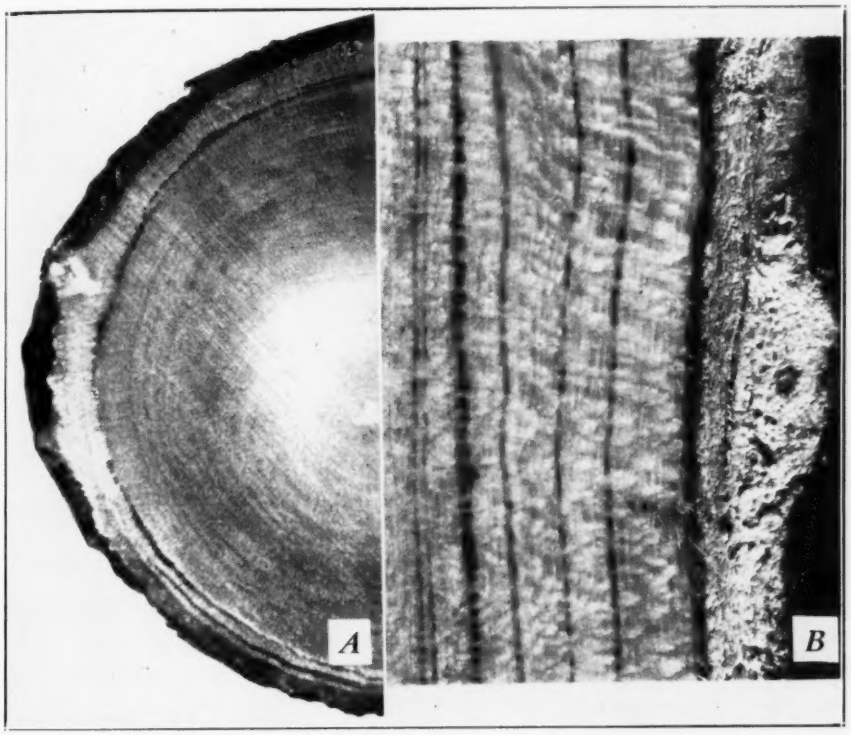

FIG. 4. Psorosis A. Wood symptoms: primary lesions. A. Transverse section of orange branch, showing overlapping gum layers at the periphery under a bark lesion. About natural size. B. Longitudinal radial section, showing successive gum layers under a bark lesion. × about 4.

mostly in the region of the axis of the wood rings and manifest themselves by extremely irregular discoloration of the wood, the outlines of which, in longitudinal sections, appear to be completely independent of the pattern of the wood elements, vessels, parenchyma, or medullary rays (Fig. 5, C and D). In transverse sections, however, it is often seen that the discoloration of the wood, although very irregular, is arranged in a way that suggests a relationship with the medullary rays (Fig. 5, A and B).

Two very distinct types of discolorations are generally apparent in transverse sections. The periphery of the discolored areas consists of an irregu-

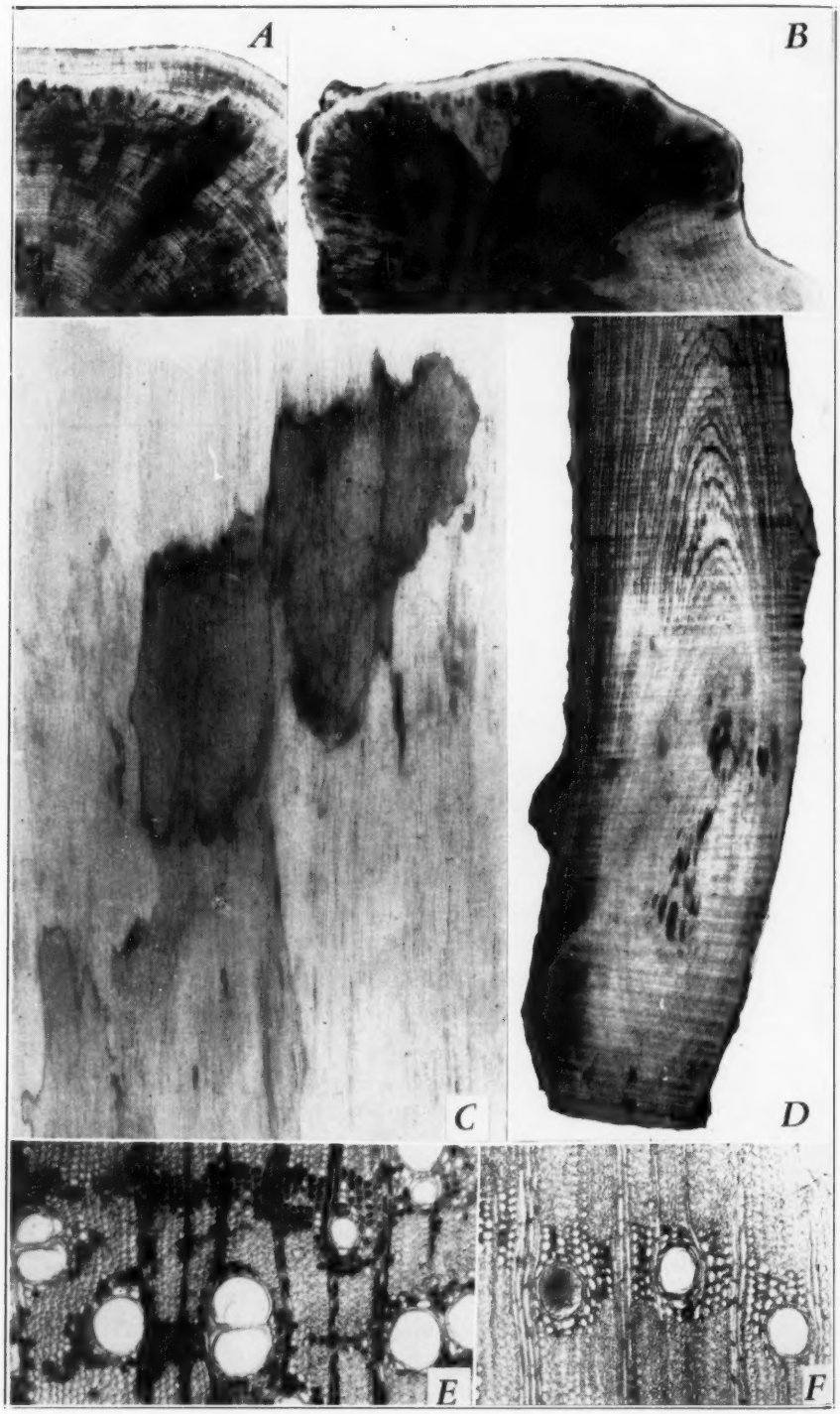

FIG. 5. Psorosis A. Wood symptoms: secondary lesions. A and B. Transverse sections of orange branches, showing wood discoloration well developed. × about ½.

lar, narrow, well-defined, orange-brown or reddish-brown line. Within this well-marked limit, the wood is irregularly stained, the predominating colors being drab to brown. In portions of the lesion, however, the marginal orange-brown line may be very faint or even entirely lacking, and the wood discoloration may gradually fade into the normal wood color at the periphery of the lesion.

The difference between the two types of discolored wood is well emphasized by examination in the light of an ultraviolet lamp, in which both are distinctly fluorescent. The narrow, sinuous marginal line has an orange-colored fluorescence especially visible in fresh material. The central, irregularly discolored portion is light purple or lilac. The fluorescence may be almost completely absent in the most central parts of the discoloration and is extremely intense in the periphery, especially when the marginal line is absent. This suggests that the latter is formed later than the central discolored area, and that it might be the result of a wood reaction tending to seal off the altered wood from the still normal wood.

In very advanced cases, the wood alterations are more pronounced; this is usually due to the invasion of the wood by secondary wood parasites. These more pronounced alterations are no longer typical of psorosis, their characteristics varying according to the nature of the secondary parasites concerned. Ordinarily, such alterations are definitely connected with some injury, such as the pruning of a limb, in the region of the psorosis lesion. The upper surface of horizontal limbs is apparently a region of stress that is exposed to different natural or man-made injuries, which open up the way for wood-invading fungi.

The wood discolorations that have just been described are accompanied by the formation of gum or gumlike substances in the wood tissues. Part of the color is probably due to the gum itself. This gum is especially abundant in the peripheral orange-colored line, where it pervades all tissues, vessels, fibers, parenchyma, and medullary rays. If a tangential section of the wood in the region of this line is observed with a powerful hand lens, the medullary rays are seen to stand out distinctly as a result of their deeper orange coloration. This would seem to indicate that there is more gum formed in the medullary rays than in the other elements of the wood. This is not the case, however, because in transverse sections the medullary rays actually appear lighter in color than the other elements, especially the wood fibers. This difference in the aspect of the wood elements in the two kinds of sections is the result of viewing the gum-filled elements endwise in tangential sections and sidewise in radial and transverse sections. The orange-colored gum pervades, indistinctly, all the wood elements in the region of

C. Longitudinal radial section of an orange trunk, showing well-developed wood discoloration. × about $\frac{3}{8}$. D. Longitudinal section of orange branch treated with I–KI, showing starch present in the normal wood and a starch-free region surrounding the wood discoloration. × about $\frac{1}{2}$. E. Transverse section from normal wood, showing starch. × about 75. F. Wood from region near discolored wood, showing no starch and a vessel plugged with gum. All vessels had gum plugs (see accompanying paper [2], Fig. 1). × about 75.

the marginal line. In the central portions of the lesions, there seems to be less gum formed than in the marginal line, and the nature of the gum seems different, as shown by the differences in coloration when sections are stained with the usual gum reagents such as phloroglucin, orcinol, or Sudan IV. Both gums are insoluble in hot water, however.

When I–KI (iodine-potassium iodide) is applied to the secondary wood lesions, the reaction shows that there is very little or no starch in the discolored portion of the wood and, further, that starch is absent, or is very much reduced, in a more or less extensive region around the discolored portion, which is not otherwise differentiated from the normal wood (Fig. 5, D). This starch reaction establishes a usually well-defined boundary outside the stained portion and probably indicates the outer advancing margin of the altered wood. It will be seen later that the starchless area, which is undetectable in macroscopic appearance from the normal wood, will not allow the passage of water and therefore contributes to decline of the affected trees in the same way as the wood that has already become discolored (2, 12).

In microscopical transverse sections of normal wood treated with I–KI reagent, starch grains are chiefly distributed in three regions—in the medullary rays and in the metatracheal and paratracheal parenchyma (Fig. 5, E). In these same regions of the discolored portion of the wood, hardly any starch grains are seen, and there are only a very few in the contiguous region (Fig. 5, F), in which there is no macroscopical starch reaction with the I–KI test.[6] In this uncolored but starchless region, no gum can be detected macroscopically; but in transverse sections, and especially in longitudinal sections, it is seen that a sufficient amount of gum is formed in different parts of the vessels to explain the lack of passage of water in the experiment described in the succeeding paper (2). This gum probably originates from the starch (1), and its formation may, therefore, explain the decrease in the presence of starch.

Before the secondary symptoms of the wood are evident, the top growth and foliage of the tree are usually normal. After the secondary symptoms develop, the tree deteriorates rapidly, leaves become small, yellow, and few in number, and twigs begin to die back. The degree of decline is probably nearly proportional to the degree in which the starch-free discolored wood and its contiguous region of starch-free nondiscolored wood occupy the whole region of water-conducting tissue. It is possible, however, that other factors, such as nutrition or toxic products, may also play some part in deterioration of the tree (2).

Leaf, Twig, and Fruit Symptoms

Young-leaf symptoms common to all the varieties of psorosis (Fig. 1) have been described earlier in this paper. Occasionally, in severe cases of psorosis A, the flecks or cleared areas on the leaf will persist for a while after the leaf begins to mature. Occasionally, also, circular spots similar to those

[6] The specimens examined were collected during the months of January to May.

described under psorosis B develop on mature leaves. Twig lesions like those described under psorosis B also develop with psorosis A, but much less frequently than with psorosis B; they usually are produced only by virulent strains of the virus. Fruit spots and sunken furrows or rings, such as those described under psorosis B, are rare.

PSOROSIS B

Psorosis B, which is caused by the virus *Citrivir psorosis* var. *anulatum*[7] (7, 8), differs from psorosis A mainly in the production of symptoms on mature leaves and on small twigs and fruit, together with the greater extent and more rapid development of the bark lesions. The young-leaf symptoms are perhaps less common, but they are similar to those of psorosis A. Although psorosis B is more deleterious to affected trees than psorosis A, the number of cases is comparatively few.

Bark Symptoms

Bark scaling on the trunk and larger limbs, with psorosis B, is similar to that of psorosis A, but gumming is more profuse in advance of scaling and less profuse after scaling occurs. Psorosis B also advances more rapidly than is usual with psorosis A, generally along one side of a trunk or branch (Fig. 6, A and B) and even into much smaller, more recently formed limbs and twigs. Elongated strips of bark may be killed through to the wood on trunk or limbs, and twigs are killed rapidly. Frequently, the outer bark is killed so rapidly that larger, longer flakes or scales are formed than at other times. Such large scales, however, may occur also with psorosis A.

Gumming, splitting of bark, and scaling of twigs of all sizes throughout the tree are common accompaniments of psorosis B and help to distinguish it from psorosis A, which rarely shows lesions on very small branches or twigs.

On twigs or water sprouts growing on badly diseased trunks, spots resembling those on mature leaves, with or without rings, occur on the green bark. As the water sprout matures, the raised portions of the spots become corky, glazed, and hard, and some of the spots resemble those commonly produced in leprosis (5, 13).

Wood Symptoms

Wood symptoms, both primary and secondary, are also found in psorosis B (Fig. 6, C), but the time between the formation of the primary and secondary lesions in the wood may be much shorter than in psorosis A. No distinct differences in wood lesions of psorosis A and psorosis B have been observed.

Leaf Symptoms

On the young, tender, rapidly growing leaves, up to the time they approach the hardening stage, the symptoms are usually similar to those of

[7] *Anulatum* = with a ring. This variety so named because of the ring spots which form on the mature leaves and on the fruit.

FIG. 6. Psorosis B on orange. A. Bark lesions on Valencia-orange trunk and main branch, showing extensive scaling. × about ⅛. B. Bark lesions on navel-orange trunk, showing scaling and one strip killed to the wood. × about ⅛. C. Transverse and longitudinal radial sections, showing primary lesions as layers of gum near the periphery of the wood, and a secondary lesion as a region of discoloration deeper in the wood. About natural size. A and B photographed by L. J. Klotz.

the other varieties of psorosis but less commonly manifested. The symptoms on the mature leaves, however, are, for the most part, different from

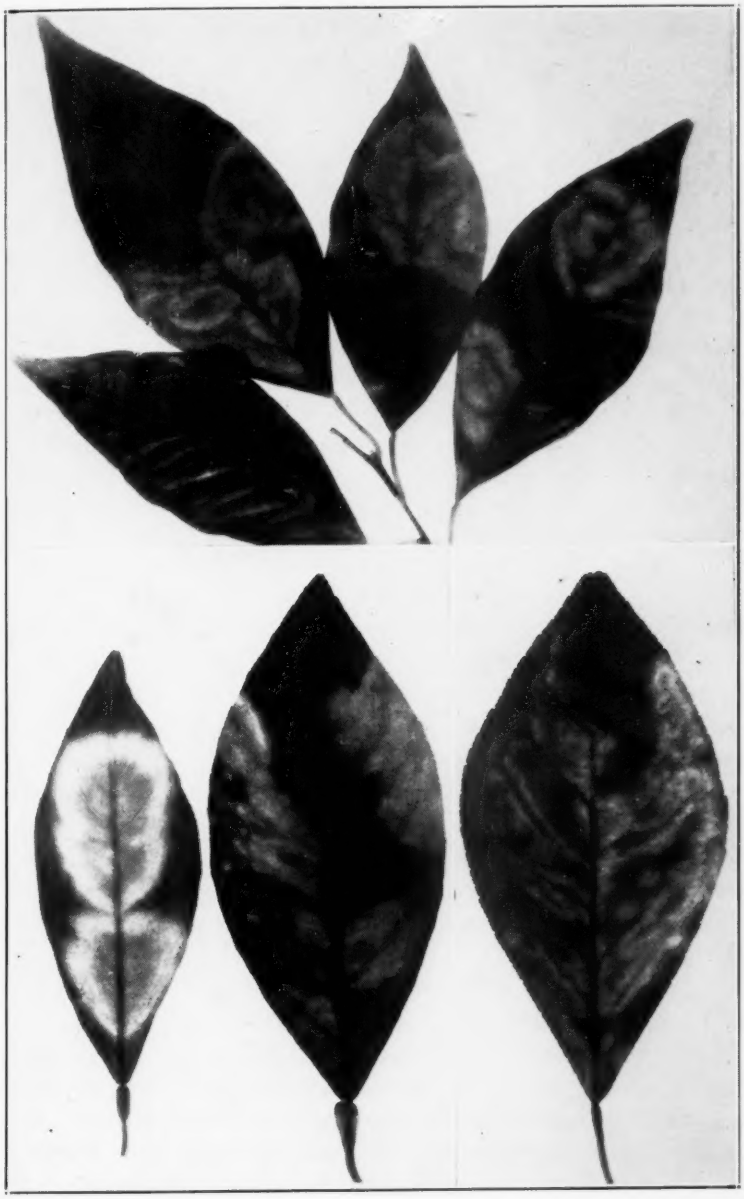

FIG. 7. Psorosis B spots on mature orange leaves, showing several patterns. × about ⅓. Top illustration photographed by L. J. Klotz.

those of any of the other psorosis varieties, except in certain severe cases of psorosis A, in which a few mature leaves may have spots resembling some of those that are common on psorosis-B trees.

The mature-leaf spots are of several kinds (Fig. 7). They range in size and appearance from mere dots to large, translucent areas, more or less circular and frequently in the form of rings. In the most conspicuous form, the spots may be large and may occur on any portion of the blade, often just above the petiole and across the entire blade. The central portion of such a spot is light to yellowish green, with or without a light-yellow circular-to-irregular border. Some spots may be in the form of single rings, concentric rings, or partial rings, occasionally with a tendency to necrosis in a portion of the rings. Another form of spot is made up of small, corky, raised areas forming a pustule or cluster of gum-filled dots surrounded by a halo and simulating certain types of leprosis (5, 13). Some of these raised dark areas are due to greater cell division in the tissue below, and to the deposition of certain products in some of the layers of cells near the surface.

In the greenish-yellow areas of mature leaves without pustules, the cells, under the microscope, appear normal in size, shape, and arrangement. In the pustular, corky areas, however, cork is produced by phellogen layers located in the palisade tissue of the upper leaf surface and in the spongy parenchyma of the lower leaf surface. The phellogen originates subjacent to cells with brown contents. Scattered groups of brown cells are sometimes seen in the mesophyll (15).

Fruit Symptoms

In some fruits of trees infected by psorosis B (very rarely in fruits of trees infected by psorosis A), surface rings bordered by sunken grooves varying in size and pattern sometimes develop. On young green fruits, circular spots similar to those on mature leaves sometimes occur. These spots are light green or light yellowish. Sometimes most of the interior of the spot remains green and is surrounded with a yellow or pale-green band or ring. A band of narrow concentric rings often occurs, accompanied by necrosis, as described for mature leaves. In other cases, especially on grapefruit, large circular grooves, partial circles, or irregular curves where rings overlap, occur on the rind and often result in a rough, bumpy condition (Fig. 8).

CONCAVE-GUM PSOROSIS

The most distinguishing features of concave-gum psorosis, which is caused by the virus *Citrivir psorosis* var. *concavum*[8] (8), are the concavities of various sizes that develop on the trunks and larger limbs. Young-leaf symptoms, similar to those of the other varieties of psorosis, are characteristic of the disease. During certain seasons, usually in the spring, the young-leaf symptoms of this variety tend to be of the zonate or oak-leaf-pattern type (Fig. 2). Although this pattern is not confined to leaves from concave-gum trees, it occurs there with sufficient regularity and uniformity during certain growth flushes to permit its use as one of the diagnostic characters. During the summer and fall, the oak-leaf pattern is usually absent.

[8] *Concavum* = concave.

Bark Symptoms

For the most part, a fairly normal bark covers the surface of concavities (Fig. 9) of varying sizes and depths on the trunk or large branches. Several concavities near together often give the appearance of a flattening of the trunk or branch (Fig. 9, A). In the central part of the concavity, or around the outer rim, cracking of the bark often occurs, with gum oozing to the surface. A small amount of scaling, somewhat resembling that of psorosis A, is occasionally associated with the development of this variety of psorosis.

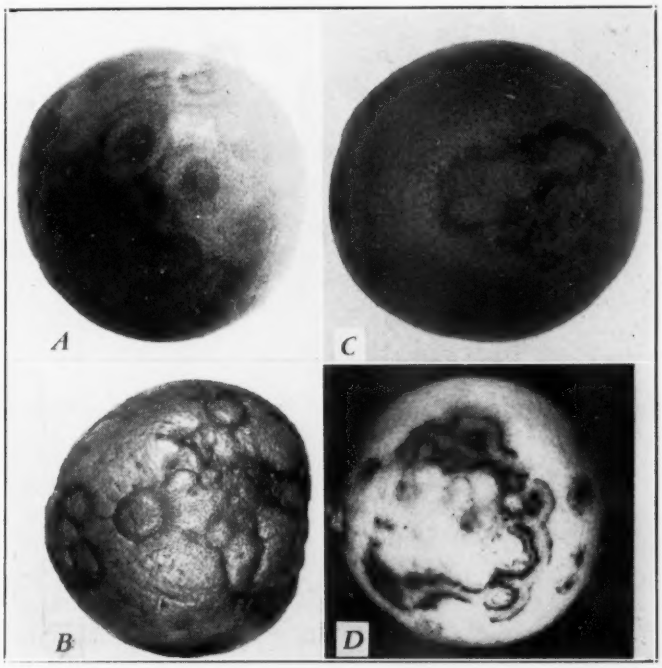

FIG. 8. Psorosis B on fruits. A and B. Depressed rings or grooves on grapefruit. C and D. Irregular grooves in the rind of oranges. × about ½. C and D photographed by L. J. Klotz.

Wood Symptoms

With concave-gum psorosis, the primary symptoms in the wood usually are limited to gum development in a very definite local area just under the concavity; such gum development is rarely extensive. As long as the concavities remain few, no secondary lesions develop in the wood, and the tree appears not to be seriously handicapped in its normal development (5). When lesions become very numerous, dwarfing and declining may occur.

The gumming in the wood vessels in the early stage of this psorosis is confined to a very small region, only a few millimeters wide in transverse section and slightly more in radial section. This region enlarges gradually as the trunk or branch produces new layers of wood, so that in the subse-

quent consecutive layers, as seen in a cross section, there is a corresponding increase in width of the wood layer in which the formation of gum takes place (Fig. 10, A). Usually, however, the gum is confined to the periphery of the altered portion of the later wood layers. In the center of the cavity, there is little or no gum formation, but the wood tissues are visibly altered and considerably thinner than in the same layer of normal wood outside the lesion. This confinement of the gum to the increasing periphery of the altered portion in the successive wood layers gives the same kind of overlapping as that occurring in psorosis A and B, previously described (compare Figs. 4 and 6), but in a more restricted way. Owing to the successive, thinner layers of wood formed at the center of the lesion, a depression is

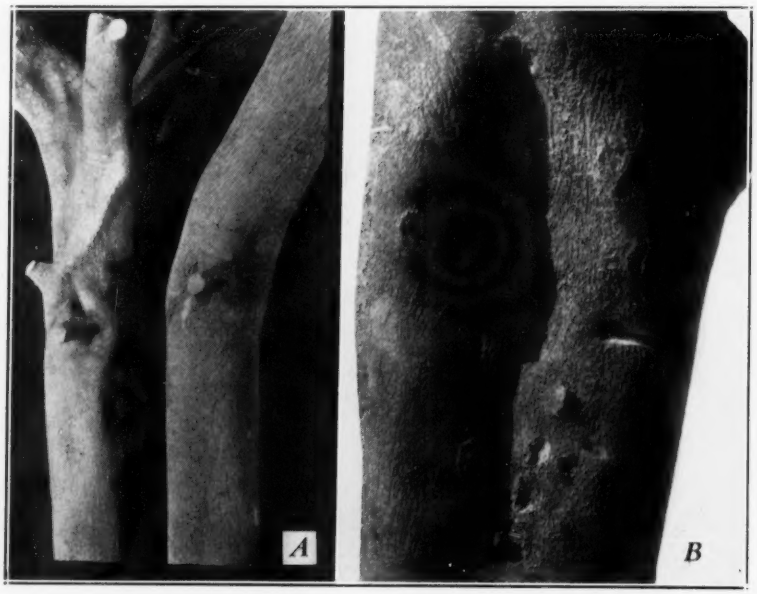

FIG. 9. Concave-gum psorosis. A. Concavities and suppressed growth in regions on orange branches. × about ½. B. Concavity on large branch, with a small amount of scaling. About natural size. Photographed by L. J. Klotz.

formed, which increases in size as the trunk or branch develops and finally produces the characteristic cup-shape concavity of concave-gum psorosis.

In the center and deepest portion of a concave-gum lesion, there frequently is found a more or less limited region where the tissues have, at some previous time, apparently been entirely inhibited, and where there is an abundance of gum. In some cases the trace of a dead twig can be detected at the same spot. In figure 10, B, which shows a cross section of a limb of sweet orange about 5 cm. in diameter, consecutive regions can be detected where extremely pronounced alterations of the wood took place during the early formation of the concave-gum lesion. The first pronounced alteration apparently took place when the limb was still quite young, and is restricted to a portion of the wood layer about 4 mm. wide in cross section. A callus

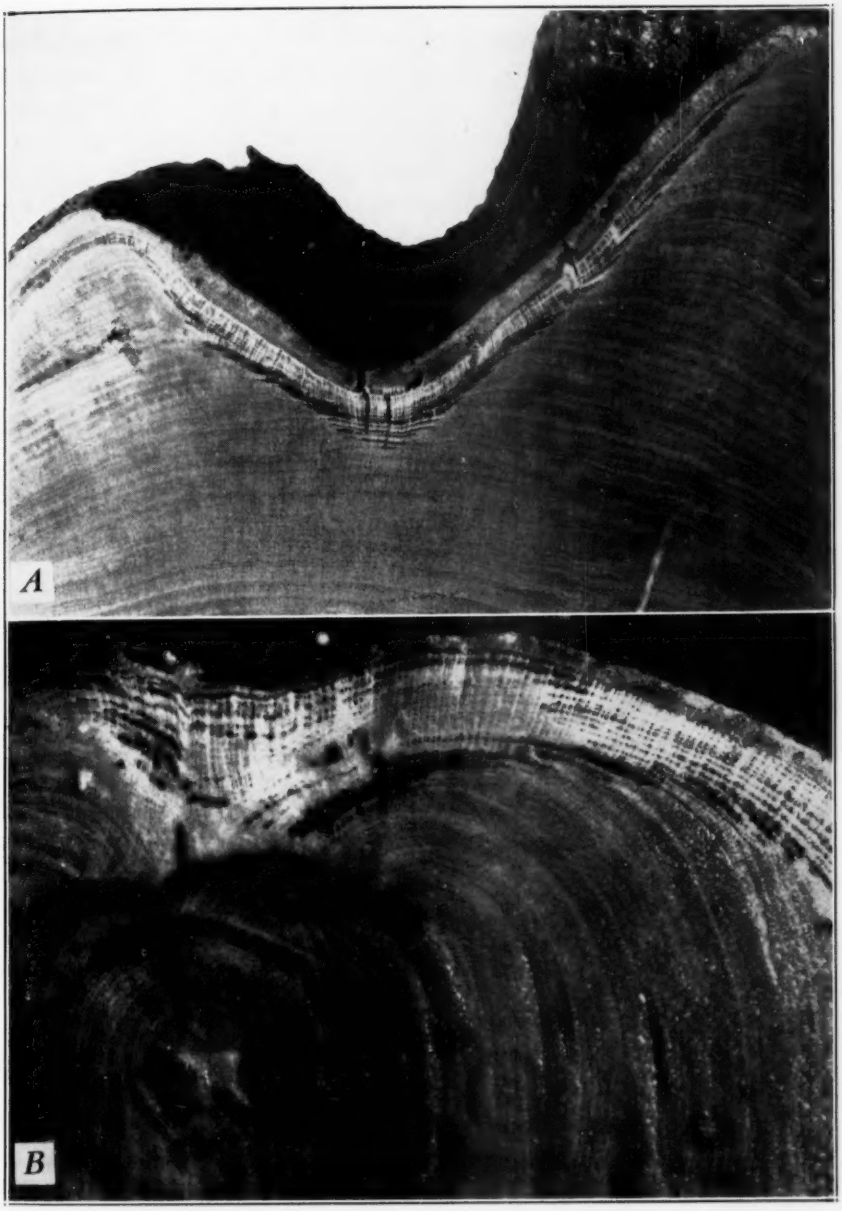

FIG. 10. Transverse sections through concave-gum concavities on sweet orange. A. Section from a lesion on the trunk, showing suppressed wood growth at the bottom of the concavity and overlapping layers of gum between layers of wood on the sides. ×1½. B. Section from a lesion on a branch, showing two regions of severe injury to wood, followed by the usual development of overlapping gum layers between wood layers and a partial recovery at the bottom of the concavity. ×3¾.

formed and covered this portion, in which the cambium either had been killed or, for other reasons, could produce no wood. Later, a new region of dead tissue, 14 mm. in width, appeared, but this region was subsequently covered with wood. From then on, gum formation took place in overlapping layers, the amount of gum diminishing substantially as new layers were formed.

BLIND-POCKET PSOROSIS

This variety of psorosis is caused by the virus *Citrivir psorosis* var. *alveatum*[9] (8). It appears on the trunk and large limbs of the tree in two forms: the most common form is a troughlike pocket in bark and wood; the other is eruptive, with bark scaling. The young-leaf symptoms are similar to those of the other psorosis varieties; no symptoms on mature leaves have thus far been observed.

Bark Symptoms

The bark in the noneruptive form of blind-pocket psorosis is usually normal in external appearance on the irregular surfaces of the narrow troughlike depressions. These lesions are often only 1 to 2 inches in length, but sometimes in older trees several lesions may run together to form much longer depressions or furrows (Fig. 11, A). The pockets usually differ from those of concave-gum psorosis in that they are narrower, more abruptly depressed troughlike depressions, frequently with two almost straight or even convex sides coming together at an acute angle at the bottom. In this angle at the bottom of the pocket or troughlike depression, the bark often is compressed or pinched by the growth of the wood on the two sides. The two sides sometimes grow together at the angle and leave a line of buried bark below. The bark often partially fills the depression and becomes thicker than the normal bark. Some cases of blind-pocket psorosis show longer or shorter depressions than others, with less abrupt slopes more nearly resembling those of the depressions of concave-gum psorosis, but distinguished from that variety by the difference in wood symptoms.

The eruptive blind pocket (Fig. 11, B) usually occurs within an area occupied by several blind pockets. It somewhat resembles bark lesions of psorosis A, for which it is easily mistaken. The scales of bark are much thicker and are usually larger than most of those of psorosis A. The possibility that such cases may be a mixture of blind-pocket psorosis and psorosis A is being investigated.

Wood Symptoms

Secondary wood symptoms consisting of internal wood discolorations are rarely produced by blind-pocket psorosis. The primary symptoms in the wood adjacent to the pockets consist in altered tissue varying in color often from yellow to ocherous-salmon. This tissue is composed of a rather loose wood parenchyma, often impregnated with either a waxy or a gummy sub-

[9] *Alveatum* = hollowed out like a trough.

stance. The apparent point of origin may be found 1 to 2 inches below the bottom of the pocket. Only rarely is gum exuded to the surface at the bottom of these pockets.

FIG. 11. Blind-pocket psorosis. A. Troughlike depressions or pockets on the trunk and main branches of an orange tree. B. Eruptive form of blind-pocket psorosis, showing thick scales of bark in region of pockets. Photographed by L. J. Klotz.

A cross section of a lesion shows that there is no considerable increase in the extent of the diseased portions of the successive wood layers; there is, however, a marked increase in the intensity of the effect. The affected por-

tion produces only an extremely thin layer of wood, as compared with the thickness of the same layer on either side of the affected portion (Fig. 12, A). In radial sections the lesion is seen to be elongated, often 3 to 8 cm. in length (Fig. 12, B). As an increasing number of wood layers are thus affected,

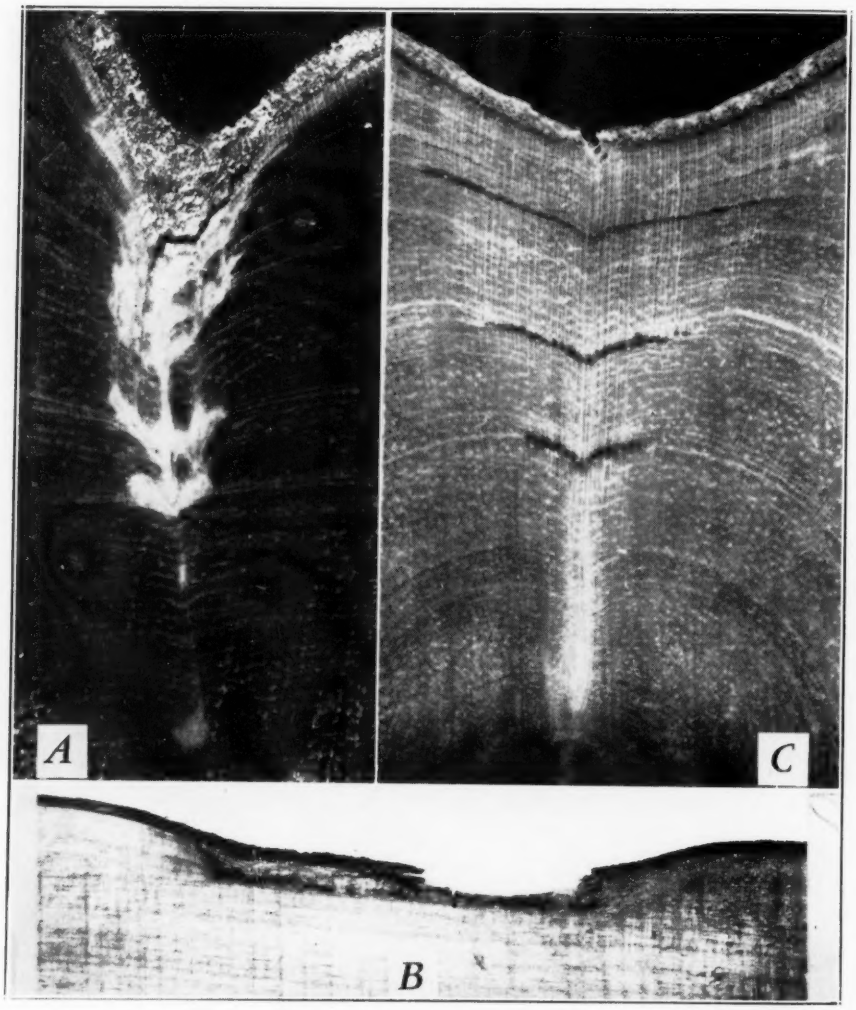

FIG. 12. A. Transverse section through a blind-pocket lesion, showing the deep origin of the pocket and the wood lesion consisting of loose parenchyma cells with local inhibition of normal wood. ×4½. B. Longitudinal radial section through one side of the pocket, the elongated region of wood inhibition about natural size. C. Transverse section through a concave-gum-like lesion. This lesion apparently began in a way typical of blind-pocket psorosis and later changed into a form characteristic of concave gum, with its overlapping layers of gum. ×4½.

a deep depression is produced, corresponding to the narrow, elongated region where the wood layers are extremely thin or are replaced by the loose wood parenchyma.

COMPARATIVE ASPECTS OF PRIMARY SYMPTOMS IN WOOD OF PSOROSIS A, PSOROSIS B, CONCAVE-GUM PSOROSIS, AND BLIND-POCKET PSOROSIS

A comparison of the primary lesions in the wood in cases of psorosis A, psorosis B, concave-gum psorosis, and blind-pocket psorosis, shows that, in a broad aspect, they differ chiefly in the extent of the alteration produced (12). In each of the 4 varieties of psorosis, the wood lesion apparently results from an alteration at or near the cambium in the affected region. For some reason not yet well understood, the cambium in that particular region ceases to produce normal wood.

With blind-pocket psorosis (Fig. 12, A and B), the alteration of the cambium is intense in its effects but limited in area. With psorosis A (Fig. 4), and generally with psorosis B (Fig. 6, C), the alteration of the cambium occupies an oval area, which progressively increases in size. The effect in the wood is a gummous degeneration of the vessels and other elements of the wood, leading to the formation of gum. With concave-gum psorosis (Fig. 10), the alteration in the wood near the cambium is intermediate between that of psorosis A or B and that of blind-pocket psorosis; although there is a progressive increase in the area affected, it is not so rapid nor so extensive as in psorosis A or B. The similarity in the nature of the overlapping layers of gum in psorosis A and in concave-gum psorosis is apparent when the figures are compared. The only real difference is the much greater extension of the layers with psorosis A or B.

This comparatively rapid extension of the layers of gum in psorosis A and B, closely following the extension of the bark lesion, eventually involves the whole, or a large area, of the circumference of the trunk, limb, or branch. When this occurs, there is a girdling effect, its severity perhaps being in proportion to the extension of the bark lesions, and to the completeness of the walls of gum within the outer wood layers.

The differences between concave-gum and blind-pocket psorosis might be considered greater than those indicated in the above comparison, because with the latter variety of psorosis, layers of gum usually are not formed in the wood. At times, however, it is difficult to distinguish between the lesions of concave-gum and blind-pocket psorosis, especially when conditions are more or less intermediate between the two extreme cases described. One such intermediate case is shown in figure 12, C; it was at first difficult to decide whether this was blind pocket or concave gum. The lesion apparently started in a way typical of blind pocket, but after a number of layers of wood had been formed without appreciable increase in the size of the affected portion, growth continued in a way usually observed in connection with concave gum.

Figure 12, C, shows that these gum layers are generally more pronounced in the portion of the wood layers that are formed early in the spring, where, in specimens of healthy wood, the vessels are more numerous and of greater lumen, and where the starch-filled wood parenchyma is more abundant.

The similarity in the primary lesions of the wood in psorosis A, psorosis B, concave-gum psorosis, and blind-pocket psorosis is a further evidence of the relationships between those diseases, already indicated by the similarity of their young-leaf symptoms.

CRINKLY-LEAF PSOROSIS

Crinkly leaf (5, 13), seen principally on lemons, appears associated with psorosis in the following ways: If sweet orange is used as the rootstock, the orange stock of the affected tree, as it becomes old enough, will have bark symptoms, usually of psorosis-A type, while the young lemon leaves of the top, as well as the young orange leaves from rootstock suckers, will have both crinkly leaf and the young-leaf symptoms typical of the psorosis varieties. The lemon part of the tree does not develop bark lesions; neither does the lemon part develop wood lesions, unless these first originate in the sweet-orange stock and advance into contiguous lemon wood.

These crinkly-leaf and young-leaf symptoms have been transmitted by budding and bark grafting from lemon to lemon and from lemon to orange, in the same manner as has psorosis A. But, while strains of psorosis A are nearly always associated with crinkly leaf, a condition suggesting that crinkly leaf may be a symptom of psorosis A, not all strains of psorosis A are found to induce crinkly leaf on lemons.

There are at least 3 possibilities in the relationship of crinkly leaf to psorosis: (a) that it is a manifestation on lemon of the presence of a strain of psorosis A; (b) that it is caused by a mixture of the psorosis-A virus and some other virus variety; and (c) that it is due to a distinct variety of psorosis virus. In the present uncertainty as to relationship, no distinct virus name is employed.

In some cases there appears to be a greater tendency for large, longitudinal cracks, known as growth cracks, to form in the bark of affected lemon trees than in corresponding psorosis-free lemon trees, but the connection of such symptoms with psorosis is unconfirmed. The presence of shell bark (5) on lemon bark should not be interpreted as showing the presence of psorosis virus. Shell bark somewhat resembles bark symptoms of psorosis. The former is caused by the fungus *Phomopsis citri* and should not, therefore, be mistaken for a symptom of psorosis. So far as is known, crinkly leaf is not accompanied by any distinctive bark or wood symptom.

Leaf and Fruit Symptoms

The young-leaf symptoms or small cleared areas observed on immature leaves in connection with crinkly leaf on lemon, are similar to the young-leaf symptoms of psorosis on orange. The mature leaves, and sometimes the young leaves, show a warping and pocketing that appear to be due to irregularities in growth in different areas or parts of the leaf blade (Fig. 13). Thickening and dwarfing of the leaves occur frequently, but not always.

The leaf effects caused by the bud mite, or other insects, resemble but should not be mistaken for true crinkly leaf.

The expression of the crinkly-leaf symptom varies on different branches and in different growth cycles. Sometimes the leaves of one cycle show severe pocketing and crinkling, while those of the next show this but slightly.

FIG. 13. Crinkly-leaf psorosis on lemon. Leaves with variegated light areas and crinkled, warped blades; lemon fruits coarse and rough. Note flecks in young leaves (upper left) and bumps on young fruit (upper middle). × about ¾. Photographed by L. J. Klotz.

Certain branches or leaves may show it severely; others, only slightly or not at all. Orange leaves are usually resistant to the crinkly-leaf effect, although they frequently show a slight crinkling.

The fruits on lemon trees affected with crinkly leaf usually tend to be coarse and rough, the fruit surface, in severe cases, being covered with irregular bumps (Fig. 13). Only occasionally have ring spots been found associated with crinkly leaf.

INFECTIOUS VARIEGATION, ASSOCIATED WITH CRINKLY LEAF

Infectious variegation (11) is characterized by large, irregular chlorotic areas, white to yellowish white, which give the leaf blade a variegated appearance (13). This disorder has been transmitted along with the crinkly-leaf condition from lemon to lemon, from lemon to sour orange, and from lemon to sweet orange. All cases of infectious variegation seen so far on lemons in California, have been associated directly with crinkly leaf; it is possibly a phase of the latter. Striking examples of infectious variegation, from a lemon tree having crinkly leaf, are shown in figure 13. Several other crinkly-leaf strains have occasionally developed symptoms typical of infectious variegation along with the crinkly-leaf symptoms. Shoots with variegated leaves alternate with shoots showing no variegation. There is not sufficient evidence to separate, definitely, infectious variegation and crinkly leaf; the former will, therefore, be considered, tentatively, an occasional symptom associated with a crinkly-leaf complex.

OTHER DISORDERS POSSIBLY DUE TO VIRUSES

Several abnormalities on citrus, whose transmission has not yet been satisfactorily proved, but are suspected to be due to viruses, are described. It should not be inferred that, because they are included in the present paper on psorosis varieties, they have been proved to be a part of the psorosis group. It is possible, however, that further study may show some of them to belong to this group.

Corky Bark

Corky bark,[10] so termed because of the development of corklike eruptions in the bark, takes on various forms, which are accompanied by varied effects on the tree itself. Gumlike substances are usually mixed with the corklike cells of these lesions, and the wood underneath may also be affected.

Leaf symptoms similar to those due to psorosis have been observed on 3 of the following forms of corky bark, as indicated; but the observed trees of the other 2 forms were not in a suitable condition of growth to determine leaf symptoms. It has not yet been determined whether or not the leaf symptoms observed are connected with the corky-bark disorders.

Necrotic-cavity Corky Bark. This type of corky bark, found by J. C. Perry on Valencia-orange trees, resembles psorosis in some respects. The lesions are in the form of necrotic cavities, the margins of which are comprised of corky, raised tissues impregnated with hard, vitreous, gumlike material, and small scales or flakes of bark. The bark in the bottom of cavities is dead and may crack off in flakes.

Crumbly-gum Corky Bark. Corky bark of this type occurs on orange trees in irregular, eruptive, gall-like formations and ridges, in most of which gum and broken tissue together form light-brown crumbly masses extending out from the surface (Fig. 14, A). In larger, older lesions, the wood tissues

[10] The term *corky bark* was first used by J. C. Perry of the East Highland Orange Company, for what is, in the present paper, called *necrotic-cavity corky bark.*

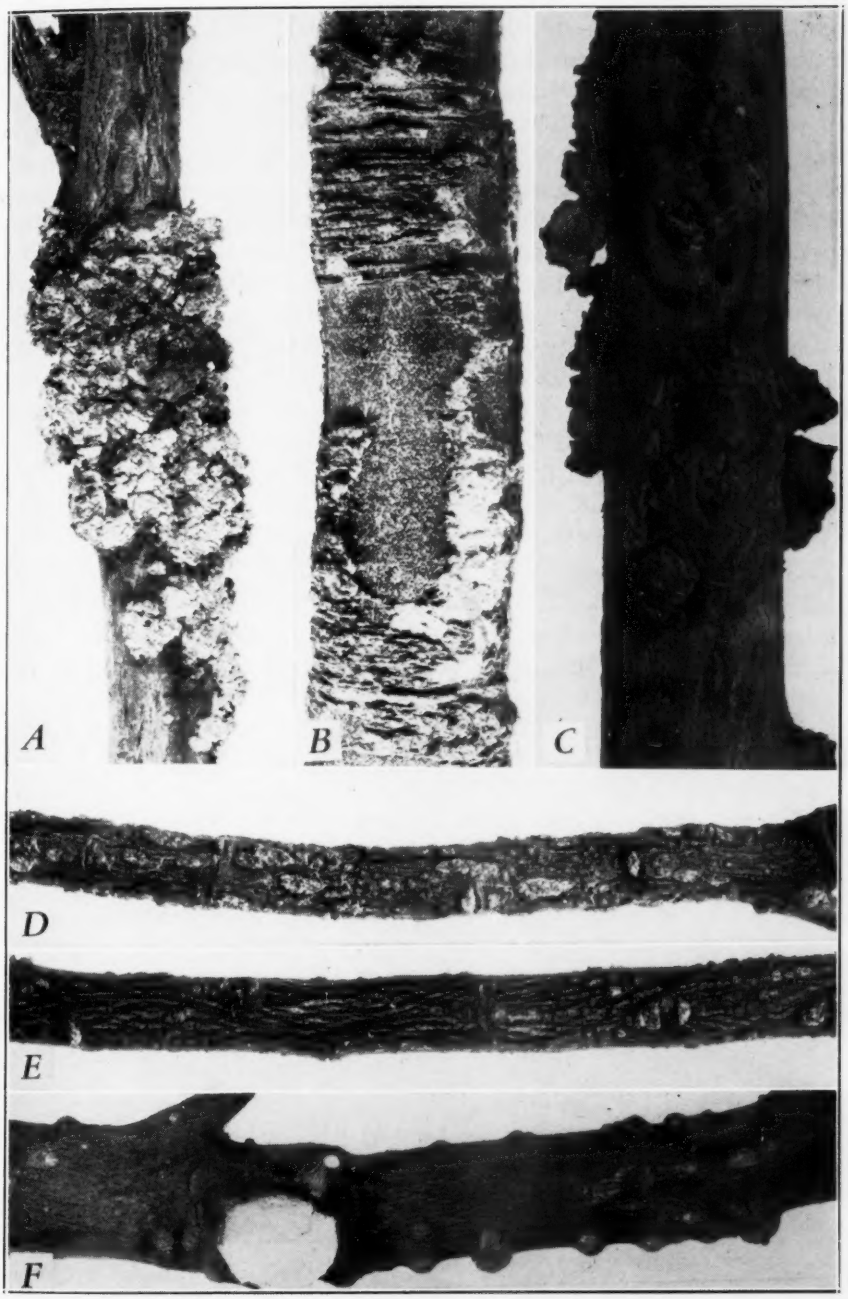

FIG. 14. Corky bark and knobby bark. A. Crumbly-gum corky bark, with erupted bark and brown gum intermingled. B. Banded corky bark on Valencia orange, showing raised horizontal bands of corklike eruptions. C. Circular-spot corky bark on navel orange, showing circular regions of erupted, corklike layers of bark. D and E. Tattoo-netted corky bark on Valencia orange, showing the irregular areas of erupted bark often suggesting a kind of tattoo. F. Knobby bark on branch of navel orange, each knoblike projection consisting of a gnarled, spherical wood interior covered with slightly altered bark. A to E, × about $\frac{3}{4}$; F, × about $\frac{1}{3}$. D and E photographed by L. J. Klotz.

contain dead portions and grow in an irregular, twisted, gnarled manner with much mixing of bark and wood in the center. Gum forms in layers in the wood as in psorosis. In some gall-like lesions, 10 to 12 mm. in diameter, abnormal wood grows into budlike projections, greenish at the periphery but necrotic within. Young-leaf symptoms similar to those of psorosis have been found on affected trees.

Banded Corky Bark. This form of corky bark, found by I. B. Suryieh on Valencia orange, appears as raised bands of corky eruptions extending horizontally around, or part way around, the trunk or limb of the tree. These may occur as narrow horizontal bands or ridges, 1 or 2 mm. wide and the same distance apart, over an area several inches long, as shown in figure 14, B. Layers of gum alternating with layers of wood occur directly inward from the bark lesion and extend somewhat beyond the margins of the lesion.

Circular-spot Corky Bark. The circular regions of erupted, corklike layers of bark formed in this type of corky bark are shown in figure 14, C. Circular-spot corky bark has been found on navel-orange trees and bears a remote resemblance to some types of psorosis A.

Young-leaf symptoms similar to those of psorosis A have been found and have been transmitted. More time is necessary to determine whether the bark symptom is also transmitted. At the periphery of the lesions, segments of corklike projections, some of which are 4 to 5 mm. in height, are formed. Each segment shows closely set layers. Sometimes the oldest part of the segment is turned back against the bark. The middle of the lesions also remains rough, the older projections having sloughed off. Other cases of circular-spot corky bark have concentric ridges of slightly raised corky tissue. Wood lesions are present beneath the bark lesions and are not unlike the primary lesions of psorosis A, with overlapping layers of gum.

Tattoo-netted Corky Bark. The irregular, elongated areas, spots, and ridges of corky bark sometimes found on trunk and limbs of Valencia-orange trees, often forming patterns (Fig. 14, D and E), suggested the name *tattoo-netted.*[11] Branch growth of affected trees often shows curved twigs and shortened nodes. Young-leaf symptoms similar to those of psorosis occur on some trees and have been transmitted by tissue fusion. Badly diseased trees often have eruptive areas similar to those of psorosis A, with primary and secondary lesions in the wood or areas resembling eruptive blind pocket.

Knobby Bark

Knobby bark[12] is a condition distinguished by the presence of knobs or hard, gall-like projections covered with nearly normal, unbroken bark. These knobs are of various sizes, ranging from 1 to 2 inches across and projecting $\frac{1}{8}$ to $\frac{1}{2}$ inch or more from the usual contour of the bark. An example of occurrence was that on a navel-orange tree, on branches 1 to 3 inches in diameter (Fig. 14, F). The bark over these knobs is about the same thick-

[11] The name first suggested for this disorder by I. B. Suryieh.
[12] Found and named by J. C. Perry of the East Highland Orange Company.

ness as in other areas. The hard, firm wood tissue underneath the knobs is more easily cut than normal wood, is light-brownish, and occurs as a spheroid core extending down into, or embedded in, the normal wood. It is separated from the normal wood by a definite margin and leaves a crater-like opening when cut out. This abnormal spheroid region of wood tissue, the knob, shows growth in circles and curves, with a light, gumlike substance within. No young-leaf symptoms were seen on trees with knobby bark.

SUMMARY

The term *psorosis* is expanded to include a group of similar disorders on citrus, previously considered to be the effects of different diseases, but now thought to be due to varieties of one virus, *Citrivir psorosis*.

The varieties of psorosis, accordingly, now include psorosis A, psorosis B, concave-gum psorosis, blind-pocket psorosis, and crinkly-leaf psorosis.

All these varieties of psorosis have, in common, a mosaiclike symptom in young leaves, characterized by white to yellowish flecks in the region of the veinlets or cleared bands along the veins and veinlets. These varieties also have fundamentally similar alterations in the wood producing the primary symptoms.

Psorosis A and B, caused by *Citrivir psorosis* var. *vulgare* and *C. psorosis* var. *anulatum*, respectively, show the following symptoms in addition to young-leaf symptoms: (a) bark lesions, characterized by a scaling of the outer bark in dry, irregular flakes, or by erumpent pustules; and (b) wood lesions of two kinds: primary lesions near the cambium, consisting of layers of gum between layers of wood directly under the bark lesions; and secondary lesions consisting of discolored wood, usually farther inward. The regions of the secondary lesions and contiguous regions of nondiscolored wood are devoid of starch and are impermeable to the passage of water.

Psorosis B differs from psorosis A in having more rapidly developing and more continuous areas of bark scaling, in having numerous twig lesions, and in having, on mature leaves and on fruit, ringed spots, which rarely occur with psorosis A.

Concave-gum psorosis, caused by *Citrivir psorosis* var. *concavum*, is characterized by the development of concavities due to the inhibition, or slowing down, of wood growth in localized regions of the trunk or large branches. Gum layers formed in the wood in this type of psorosis are similar to those of psorosis A and B but more localized.

Blind-pocket psorosis, caused by *Citrivir psorosis* var. *alveatum*, usually appears as troughlike depressions in trunk or limbs, due to inhibition of wood growth in even more localized and restricted regions than in concave-gum psorosis. The sides of the depressions may be straight or convex. Underneath the lesion, the loose wood parenchyma is usually impregnated with either a waxy or a gummy substance.

The primary lesions in the wood of the 4 preceding varieties, considered

in broad aspect, have similar features and differ chiefly in the extent of alterations of the wood. In blind pocket, the alteration is intense but narrowly localized; in concave gum, less intense but somewhat spread; while in psorosis A and B, the alteration is spread out over large regions and may have even a girdling effect on trunk and limbs.

Crinkly-leaf psorosis is evident mainly on lemons and develops, in addition to the young-leaf symptoms, a warping and pocketing of mature leaves. The fruit is usually rough and coarse with irregular bumps. There are no distinctive bark or wood symptoms.

Infectious variegation, which is characterized by irregular chlorotic areas on the leaf blade, appears occasionally on lemon leaves. Since there is not sufficient evidence to separate infectious variegation and crinkly leaf, the former is tentatively considered an occasional symptom associated with a crinkly-leaf complex; both, when they occur, are usually found associated with psorosis A.

Other possibly virus effects, which may be related to the preceding psorosis varieties, are corky bark and knobby bark. Five kinds of corky bark have been observed: (a) necrotic-cavity, (b) crumbly-gum, (c) banded, (d) circular-spot, and (e) tattoo-netted.

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THE RELATIONS OF WOOD ALTERATIONS IN PSOROSIS OF CITRUS TO TREE DETERIORATION[1]

A. A. Bitancourt,[2] H. S. Fawcett,[3] and J. M. Wallace[4]

(Accepted for publication March 3, 1943)

INTRODUCTION

In a description of the symptoms of the different varieties of the psorosis of citrus, Fawcett and Bitancourt (6) point out that in psorosis A and psorosis B, caused by the 2 virus varieties *Citrivir psorosis* var. *vulgare* and *C. psorosis* var. *anulatum*, respectively (4, 5), there are, in addition to bark lesions, 2 types of wood lesions, primary and secondary.

The primary lesion forms in the wood soon after and almost coextensively with the lesion in the bark, and is characterized by gum layers between layers of either normal or altered wood. The secondary lesion appears to begin in older, more interior wood only after a considerable period of time, usually several years after the inception of the bark lesion and the primary wood lesion. The secondary lesion is marked by an irregular discoloration of the inner wood (3), with wood vessels partially or entirely filled with gum, the amount depending on the stage of development of the lesion, and by an absence of starch in both the discolored wood and a contiguous border surrounding the discolored area. The starch-free border follows almost exactly the pattern shown by the outer margin of the discolored part of the wood and also shows gum-plugged vessels. However, most wood vessels in the tissues contiguous to the discolored wood show accumulations of small quantities of gum on one or both sides of the perforation plates, as described earlier by Webber and Fawcett (8). This type of plugging is shown in figure 1, B and D. The gum plugs seem to be contemporaneous with the disappearance of starch and probably are derived from it (2). This secondary lesion of the more interior wood may finally extend until it includes the primary lesion near the bark. The degree of involvement of all the wood appears, in general, to be correlated with the degree of deterioration of the tree or part affected.

These wood alterations are accompanied by retarded growth and progressive deterioration of the branches and foliage. The growth of new twigs is less vigorous, the foliage becomes gradually less dense, the leaves become smaller and fall prematurely, and the twigs usually die back after many

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leaves have been shed. Occasionally, individual branches die rapidly, and the leaves dry before they are shed. Even when the branch or trunk dies back to the discolored region, the roots remain alive and tend to send up new shoots.

Observation of many citrus trees has shown that the psorosis bark lesions and the primary lesions in the wood may be present for a considerable time

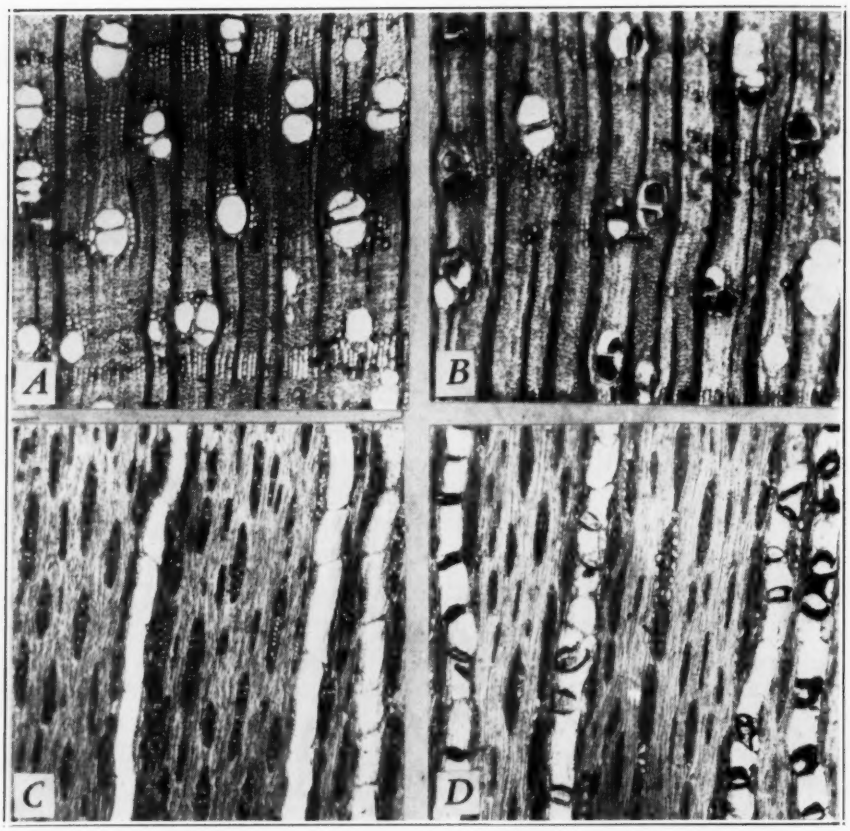

FIG. 1. A. Transverse section of wood of healthy orange tree. ×40. B. Transverse section of wood of psorosis-affected orange tree, showing gum plugs in xylem vessels in tissues contiguous to secondary wood lesion. ×40. C. Tangential section of wood of healthy orange tree. ×40. D. Tangential section of wood of psorosis-affected orange tree, showing accumulation of gum at perforation plates of xylem vessels. Sectioned from wood free of discoloration but near secondary lesion. ×40.

(several years in most cases) before any marked deterioration in growth or foliage takes place. It is only after the secondary wood lesions appear, with the visible discoloration of the wood in the interior of the trunk or branches, that noticeable deterioration takes place.

This paper presents the results of a study of wood alterations in relation to deterioration of psorosis-affected citrus trees.

WATER-CONDUCTION EXPERIMENTS

The investigations of Webber and Fawcett (8) and of Fawcett and Bitancourt (6) disclosed that the xylem vessels of tissues in the vicinity of psorosis lesions were commonly filled or partly plugged with gum. This suggested that the deterioration of affected trees might be due to an insufficient conduction of water through the affected parts to the upper portions of the trees. Experiments were undertaken to determine the effect of primary and secondary lesions on water conduction through citrus wood.

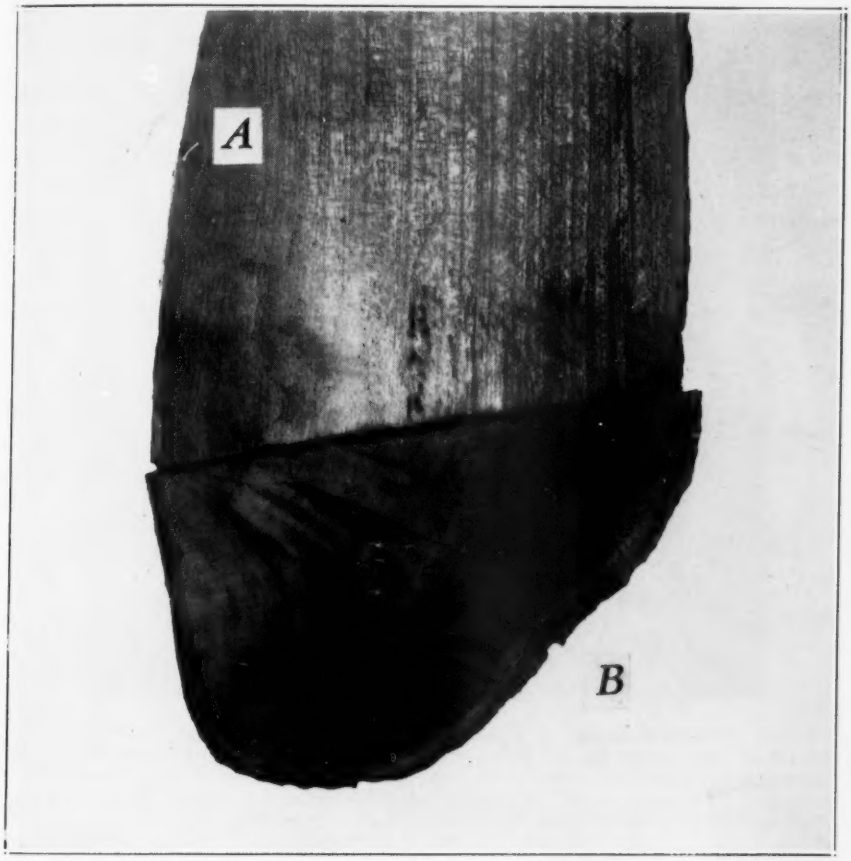

FIG. 2. Longitudinal (A) and transverse (B) sections of a block of orange trunk wood having a secondary lesion in the wood. The lower portion of the block had been dipped in an 0.5 per cent aqueous solution of safranin for a period of 7 hours. Later, the whole block was treated with the I–KI test for starch. Safranin stain, which penetrated the starch-containing regions of the healthy wood, is seen as the darkest area in the lower right portion of the longitudinal section (A) and as the darkest area in the transverse section (B). The irregular, starch-free, discolored areas of the secondary lesion, at the left, are shown surrounded by the lighter, starch-free portions of the lesion. Natural size.

Preliminary Experiments

In preliminary experiments, the regions of the wood where the passage of water is likely to take place in diseased specimens, were determined by

the staining of wood elements with 0.5 per cent aqueous solutions of acid fuchsin or safranin.

Figure 2 shows longitudinal and transverse sections of a block of trunk wood, the lower end of which was maintained in a solution of safranin for over 7 hours. The solution was absorbed into the wood by capillarity and penetrated the wood vessels approximately $1\frac{1}{2}$ cm. In some vessels, however, the stain penetrated up to 4 cm. When the I–KI (iodine-potassium iodide) test for starch was applied to the same section, it was seen that the

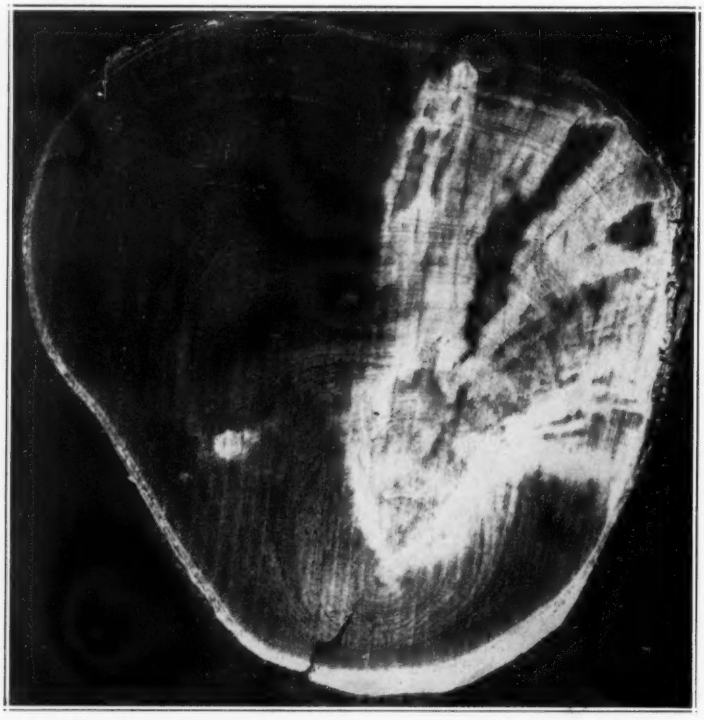

FIG. 3. Tansverse section of an orange branch, showing secondary lesions. The continuous dark portion (at the left and bottom) shows the presence of starch in the unaffected wood, as determined by the I–KI test. The irregular dark patches surrounded by white constitute the regions of the secondary lesions. Starch is absent or slight in these regions, and the passage of water is blocked. About natural size.

regions that the safranin solution had failed to penetrate were those showing little or no starch present. These were the regions of the visibly affected secondary lesions and the starch-free regions around them. This indicated that water passage was being interfered with in the discolored portion of the wood of the secondary lesion and also in a contiguous region, which could be revealed only by the absence of reaction in the I–KI test for starch. For example, in a section such as that shown in figure 3, treated with I–KI, water can pass in the black, starch-bearing portions of normal-appearing wood at the left and at the bottom, but is blocked in the remaining nearly starchless

portions of the secondary lesions, which show as white areas intermixed with irregular dark patches.

In other experiments, a solution of acid fuchsin was drawn into the cut branch or wood block by dipping one end into the solution while maintaining a partial vacuum at the other end.[5] In most experiments, sections of branches, or wood blocks, 5 cm. long were used.

These tests, like those of Villiers (7), indicated that in normal, healthy wood there was very little lateral movement of the stain. Most of the passage of aqueous stain took place in the peripheral region of the wood. The smaller, central portion allowed less stain to pass. The visibly affected wood allowed no stain to pass, and the adjacent wood usually acted in the same manner. For example, figure 4, A, shows the movement of the acid fuchsin stain in a block 7½ cm. in diameter and 5 cm. high, taken within close proximity to a discolored region of a secondary wood lesion. The effect of the I–KI test for starch is shown in figure 4, B, a section cut from wood contiguous to that shown in figure 4, A. The region of passage of acid fuchsin solution is seen to be identical with that of the positive starch reaction. This experiment also showed that passage of water was blocked in the discolored wood of the secondary lesion and in the contiguous starch-free wood. It may be concluded that the conducting vessels in these portions had been clogged.

TABLE 1.—*Time required for passage of the first 3 cc. (of 8 cc.) of water through each of three portions of a healthy block of wood 5 cm. in length, in five successive runs, under suction equivalent to four-fifths of an atmosphere*

Location in block	Successive runs				
	1	2	3	4	5
	Seconds	*Seconds*	*Seconds*	*Seconds*	*Seconds*
a	42	48	56	75	103
b	26	30[a]	35	55	75
c	53	58	70	107	130

[a] Interpolated.

In order to obtain a measure of the extent to which the wood alterations interfere with the passage of water, experiments were made in which a given number of cubic centimeters of water were drawn through specific portions of 5-cm. sections of branches or blocks of trunks and large limbs of affected trees. One end of the block was placed in water; at the other end a suction equivalent to approximately four-fifths of an atmosphere was maintained.

It is known that the amount of water or other fluids that can be passed through wood by forcing the fluids under pressure, or by suction at one end, the other end being immersed in the fluid, is extremely variable (1). There

[5] In this and following experiments, one end of the cut branch or wood block was dipped in water or stain solution, the free surface of which was, of course, at atmospheric pressure. A suction corresponding to approximately 60 cm. of mercury was then applied to the other end. This suction produced a difference of approximately four-fifths of an atmosphere of pressure.

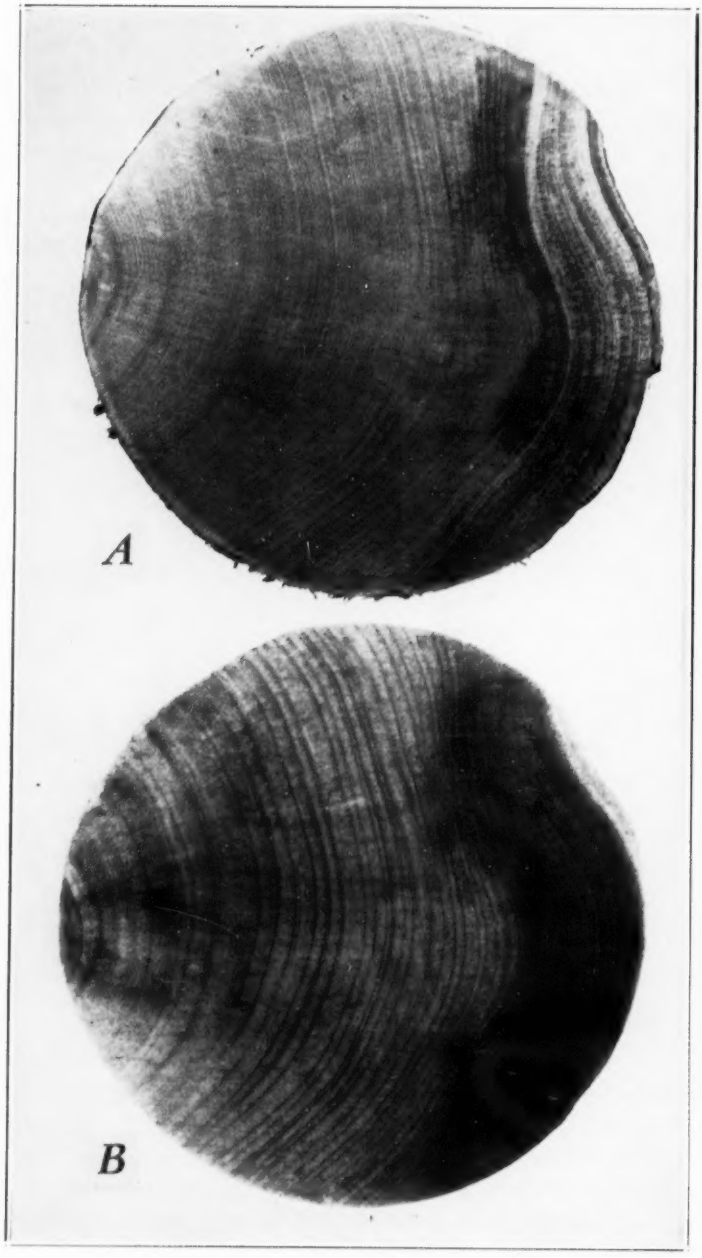

FIG. 4. A. Transverse section of a block of orange trunk wood taken slightly above a region showing the discoloration of a secondary lesion. The wood next to the bark shows staining resulting from an aqueous solution of acid fuchsin, which was drawn through the wood by suction. B. Transverse section of a block of trunk wood taken from the same tree, close to the preceding block. This wood was treated with I–KI and shows that the starch-containing region corresponds with that through which the fuchsin solution passed. The lighter, nearly starch-free region in which the passage of the fuchsin was blocked, as shown in A, was clogged with gum plugs as a result of its proximity to the secondary wood lesion. Natural size.

is a considerable decrease in the rate of flow with time, even during the short period involved in experiments in which only a few cubic centimeters of fluid are passed through the wood.

The results of one experiment with a healthy block of wood, 7½ cm. in diameter and 5 cm. in length, are presented in table 1. In this experiment the time required for the first 3 cc. (of 8 cc.) of water to pass through each of 3 specific portions of the block, in 5 successive trials, was determined. The water was drawn through at 3 different places, each approximately 1 sq. cm. in area, on the surface of the block, near the bark. A difference of about four-fifths of an atmosphere was maintained in the pressure at the two ends of the block. It is apparent that the time required for water passage consistently increased with each successive trial, until in the fifth trial the time required was between two and three times that of the first trial. This table also shows that there is a considerable variation in the amount of water that can be drawn through different but similar portions of the same block of healthy wood, in a given period of time. There is also a distinct relationship between the amount of water passing in a given time and the width of wood layers: the wider the wood layers, the larger the amount of water passed. The difference between narrow and broad wood layers is shown in figure 6, A.

Water Conduction in Wood Having Primary Lesions Only

Results of an experiment in which water was passed through two 5-cm. sections cut from a psorosis-infected branch 2.5 cm. in diameter, are shown in table 2. One of the sections had a bark lesion, with its accompanying

TABLE 2.—*The effect of bark and primary lesions on the rate of water passage, under suction equivalent to four-fifths of an atmosphere, through two sections of wood, each 5 cm. long and about 2.5 cm. in diameter, cut from the same branch of a psorosis-infected tree (experiment conducted as shown in Fig. 5)*

Amount of water passed	Wood without lesions			Wood with lesions		
	Successive runs			Successive runs		
	2	3	4	2	3	4
cc.	*Seconds*	*Seconds*	*Seconds*	*Seconds*	*Seconds*	*Seconds*
1	23	42	49	61	154	225
2	65	84	111	119	291	505
3	106	140	171	194	445	740

primary wood lesion; the other, cut beyond the lesions on the same branch, was lesion-free. Here again, the time required for the passage of equal amounts of water increased with each successive trial, the fourth trial requiring one and one-half to twice as much time as that of the second run in the lesion-free section. The fourth trial with the lesion-affected section required about 4 times as much time as the second trial with this same section. Although there was no evidence of a secondary lesion in the lesion-

affected block, the difference in the rate of water passage through the two blocks, throughout the experiment, was very marked. However, even though water was retarded by the bark lesion and primary wood lesion, the reduction in water passage was not sufficient to cause marked deterioration in such a branch.

This experiment shows that, if the amounts of water that can be passed through different specimens of citrus wood, or even different parts of the same specimen, are to be compared, not only should the same suction be applied, but other factors, such as the places where the suction is applied, the amount of water to be drawn, and the amount of water just previously drawn through, should be taken into consideration. In order to avoid the errors due to the extremely variable rate of flow resulting from these factors, most of the following experiments were made comparatively. Comparable sections of branches or blocks of wood, healthy and diseased, were collected on the same occasion and were cut and prepared in the same way. Water was drawn through these sections simultaneously, in the same apparatus, with the same differences in pressure.

TABLE 3.—*The effect of bark and primary lesions of psorosis on time required for passage of 10 cc. of water, under suction equivalent to four-fifths of an atmosphere, through sections of Valencia-orange wood, 5 cm. in length and 2 to 3 cm. in diameter*

Date, 1942	Experiment No.[a]	Wood without lesions	Wood with lesions
		Seconds	*Seconds*
May 11	1	20	32
	2	22	30
	3	28	24
May 13	4	31	64
	5	43	66
	6	57	44
July 14	7	19	25
	8	55	129
	9	63	82
Aug. 12	10	10	55
	11	17	80

[a] Each experiment involved a pair of sections, one cut from a portion of a branch showing bark lesions of psorosis, the other from an unaffected portion of the same branch or set of branches. Experiment conducted as shown in figure 5.

Numerous experiments on the passage of water through psorosis-affected and nonaffected wood were then carried out. In one series of experiments, sections about 5 cm. long were cut from Valencia-orange branches 2 to 3 cm. in diameter. Prepared sections were paired, one section of each pair being from a portion of the branch showing a bark lesion of psorosis A, and the other section from a portion having no bark lesions. A glass tube 2.2 cm. in diameter was sealed to one end of each section with Duco cement, and the other end was placed in water. The same suction was applied simultaneously to the glass tubes by means of a U tube, as shown in figure 5. The time required for water to pass through the sections and to rise to a 10 cc. mark

was then recorded. The time is shown for 11 pairs in table 3. In some of the experiments where suction was not applied simultaneously, the affected and nonaffected sections of the pairs were tested under approximately the same conditions of pressure, the test of one section quickly following that of the other.

In these tests, with two exceptions, water passed through those sections having bark and primary wood lesions but no indication of secondary lesions,

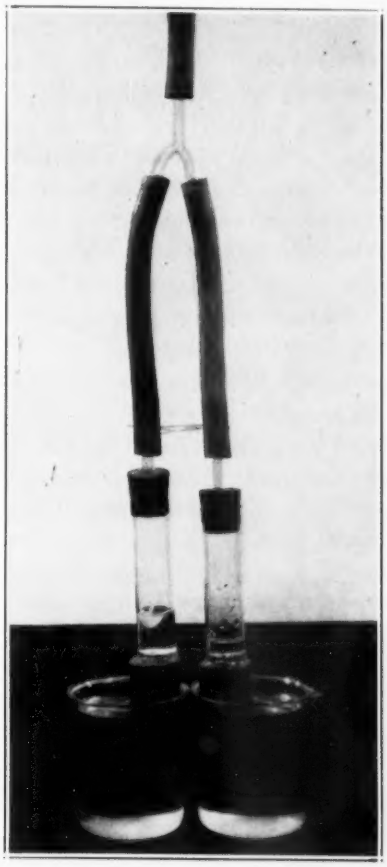

FIG. 5. Apparatus for drawing water through sections of healthy (left) and diseased (right) branches, in pairs, under the same suction. Note difference in water level under same suction.

somewhat more slowly than through corresponding sections cut from the same branch or set of branches, but without bark lesions. Although this indicates a tendency for water to be retarded in the wood vessels adjacent to or under the bark lesions, the retardation of water, obviously due to the primary lesion alone, is insufficient in most cases to cause apparent injury to foliage and fruit. This agrees with most field observations, that no marked deterioration of the tree occurs, even with bark lesions present, so

long as no interior discoloration of wood, characteristic of secondary lesions, is present. The slight or partial retardation in water passage shown in table 3 probably results from the initial gumlike deposits between the outer layers of wood and from some gum plugs in wood vessels in the primary lesions. Sufficient normal or unaffected wood is laid down to allow enough water to pass to meet the requirements of the tree.

Water Conduction in Wood Having Both Primary and Secondary Lesions

The following experiments with wood having secondary lesions of starch-free, gum-plugged wood indicate the main reason for rapid deterioration of the tree.

Experiment 1. Experimental material was taken from a psorosis-infected Valencia orange tree. Sections 5 cm. in length were cut from branches 2 to 3 cm. in diameter, showing severe interior discoloration (secondary lesions) in the wood in certain portions. Suction was applied, as in previous experiments, to each of 3 sections of a branch cut beyond the discolored wood and showing no secondary lesion. The time required for 1 cc. of water to pass through these blocks was 17, 22, and 28 seconds, respectively.

With 3 other sections, each having a secondary lesion showing advanced wood discoloration with a margin consisting of a narrow band of clear wood extending to the bark, it required 5 minutes for 0.2, 0.2, and 0.4 cc. of water, respectively, to pass through. At the same rate, it would have taken 25, 25, and $12\frac{1}{2}$ minutes, respectively, for 1 cc. of water to pass.

In another comparison from the same tree, a block with no secondary lesions showed passage of 1 cc. of water in 41 seconds, but a block with partly discolored wood of a secondary lesion required about 4 minutes for the passage of 1 cc. of water. In these comparisons, the presence of secondary lesions in the wood retarded water passage sixfold to eighty-eight fold.

Experiment 2. Similar tests were conducted on wood from a Valencia tree affected with psorosis B (6). Under a suction of 60 cm. of mercury, in two separate tests, $1\frac{1}{4}$ and $1\frac{3}{4}$ minutes were required for 10 cc. of water to pass a distance of 11.5 cm. through branches 2.5 cm. in diameter, showing bark lesions and primary lesions of the wood. In a comparative test with a branch of the same dimensions with wood showing also the discolored secondary lesions, 26 minutes were required for passage of 4 cc. of water, a rate approximately 45 times slower than that obtained with the wood not showing secondary lesions.

This comparison of time of water passage between sections having secondary lesions of discolored wood and sections having only bark lesions and primary lesions, without discoloration in the wood, appears to explain why branches having secondary lesions suffer deterioration and final decline, especially in hot, dry weather. Such branches are unable to get water fast enough in times of stress to supply the foliage and fruits. Many leaves,

therefore, drop and some twigs die back until the smaller supply of water available can take care of the remainder.

Experiment 3. Cylindrical blocks of wood, 5 cm. high and 7.5 cm. in diameter, were prepared from healthy navel-orange trunks and from trunks having interior wood discoloration. Glass tubes about 1 cm. in diameter were sealed to the upper, cut surface of each block with Duco cement and plasticine. The portion of the block between the bases of the tubes was covered with Duco cement. These blocks were then tightly placed in the end of a glass cylinder, 7.5 cm. inside diameter, with an outlet at the top fastened to the suction hose.

With a cylinder of wood from a healthy tree, showing starch over the entire cross section, the time required for 1 cc. of water to pass, in 3 regions in the section near the bark, was 26, 42, and 53 seconds, respectively. With a cylinder of wood from a diseased tree, showing starch in corresponding regions near the bark but having a secondary wood lesion some distance inward, the time required was 30, 60, and 90 seconds, respectively. In regions in the diseased wood of the latter specimen, where no starch reaction was shown by the I–KI test, no water reached the upper surface in 15 minutes.

This experiment indicates that with portions of wood from a diseased tree, even though the wood may appear normal and show the starch reaction, the time required for water passage is somewhat longer than with similar portions of wood from a healthy tree. It also shows that in the discolored wood of secondary lesions of psorosis, water passage is entirely blocked.

Experiment 4. In this experiment, glass tubes about 1 cm. in diameter were sealed with Duco cement in 0.5-cm. holes bored into sections of trunk and limbs showing the discoloration of secondary lesions. Each specimen was cut so that there would be 5 cm. of wood for the water to pass through. The time required for 1 cc. of water to pass through 5-cm. lengths of wood, at different locations in three different specimens, is shown in figure 6.

The results show that, as in previous experiments, only in the wood where the I–KI reaction indicated the presence of relatively large amounts of starch did water pass through readily. In areas of discolored wood or in areas not reacting to I–KI, no water passed when suction was applied for 10 to 20 minutes. At the edge of the discolored area in specimen C, at *f* (Fig. 6), where the I–KI test showed the presence of starch in about one third of the area covered by the tube, only about 0.1 cc. passed in 5 minutes, but no water passed through the discolored areas at *g* and *h*. In specimen B (Fig. 6), which was cut beyond the discolored portion of the limb, 1 cc. of water passed a distance of 5 cm. in the starch-bearing region (*d*) in 1½ minutes, but no water got through the starch-free wood (*e*) even after long periods of time. In specimen A (Fig. 6), from a branch of a healthy tree, with the original growth center much to one side, the rate of water passage was many times faster (1 cc. in 35 seconds), at *a*, on the side where the wood had grown more rapidly, than at *c*, on the other, slower-growing side (rate,

1 cc. in 17 minutes). Near the center, at *b*, the rate was 1 cc. in 24 minutes and 1 second.

Experiment 5. A cross section, 11 by 13 cm., of a 5-cm. block of wood cut from a trunk of a diseased tree is shown in figure 7. Water passed readily through the 5 cm. of wood at *a* and *b*, lying outside the margin of the psorosis-discolored area (1 cc. in 60 and 45 seconds, respectively). On the other hand, water either failed to pass, or else moved very slowly, through the wood closely adjacent to or within the secondary lesion of discolored wood. In one region (Fig. 7, *c*) half within and half outside the discolored

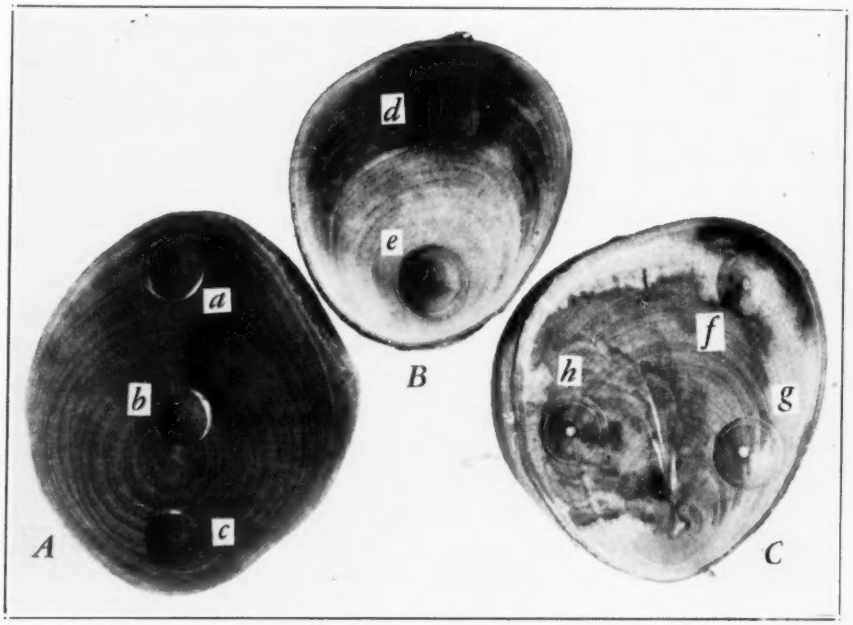

FIG. 6. Transverse sections of 5-cm. blocks of wood from Valencia-orange branches, on which graduated tubes had been attached for water suction. The regions showing the heavier black coloring are those containing starch, as indicated by the I–KI test. The central, irregularly colored region of section C is wood discoloration caused by psorosis A. A. Block from a healthy branch. The presence of starch is shown over the entire section, and water passed in all three regions, as follows: *a*, 35 seconds; *b*, 24 minutes, 10 seconds; *c*, 17 minutes. B. Block from a diseased branch, cut above but near discolored wood of secondary lesion. Starch was present only in the outer layers of wood on one side of the branch, where 1 cc. of water passed (*d*) in 1 minute, 30 seconds. No water could be drawn through the starch-free area (*e*). C. Block from the same branch as B, but containing psorosis-discolored wood. Wood containing starch was confined to a very small portion (upper right). It required 50 minutes for 1 cc. of water to pass through this region at *f*. No water passed through the other two regions (*g* and *h*) of discolored wood where no starch was indicated. All × ⅔.

wood, it took 24 minutes for 1 cc. of water to pass. The regions *d*, *e*, *f*, and *g* (Fig. 7), occupied by the psorosis wood lesions, allowed no water to pass in 24 minutes or more, under the same suction.

The results in experiments 4 and 5 (Figs. 6 and 7) show, further, that the passage of water appears to be completely prevented in the region of the

discolored secondary lesions. They also show, as have some of the previous experiments, that outside of the discolored region, absence of starch appears to be coincident with the regions where passage of water is partially or totally blocked. This blocking of the supply of water would, in part at

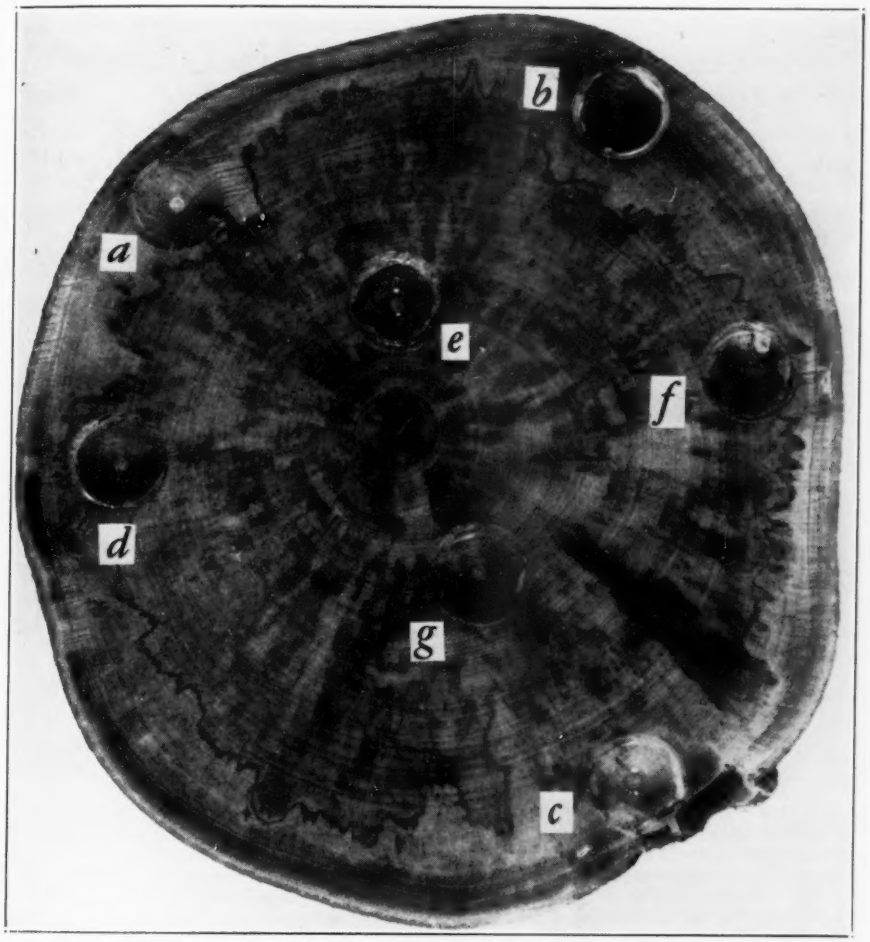

FIG. 7. Cross section of a block of wood from a diseased Valencia-orange trunk cut beyond the bark lesion but showing severe secondary lesions of the wood. The time required for 1 cc. of water to pass, under a pressure of four-fifths of an atmosphere, through this 5-cm. block, in which the I–KI test showed the presence of some starch, was as follows: *a*, 1 minute; *b*, 45 seconds; *c*, 24 minutes. These three regions were partially or wholly outside the psorosis discoloration. In four other regions (*d*, *e*, *f*, *g*), all within psorosis-discolored wood, no water could be drawn. The top of the tree from which this block was cut had only partially deteriorated.

least, account for the gradual deterioration and decline of trees having well-developed secondary lesions in the wood, and would account for the rapid deterioration where the secondary symptoms involve more nearly the entire wood in some part of a trunk or limb.

AIR PASSAGE THROUGH BARK

The question also arose as to the possible effect of psorosis lesions on the amount of air that can pass through the bark. A simple experiment showed that more air passed from the wood through the area occupied by the bark lesion, especially at the margin, than through the uninvolved bark just beyond.

Several branches, about 2.5 cm. in diameter and 15 cm. in length, were used in these experiments. Each branch showed a bark lesion of psorosis A completely girdling one of its extremities. The affected end was carefully sealed with an air-tight rubber cap. The other end was sealed into a glass

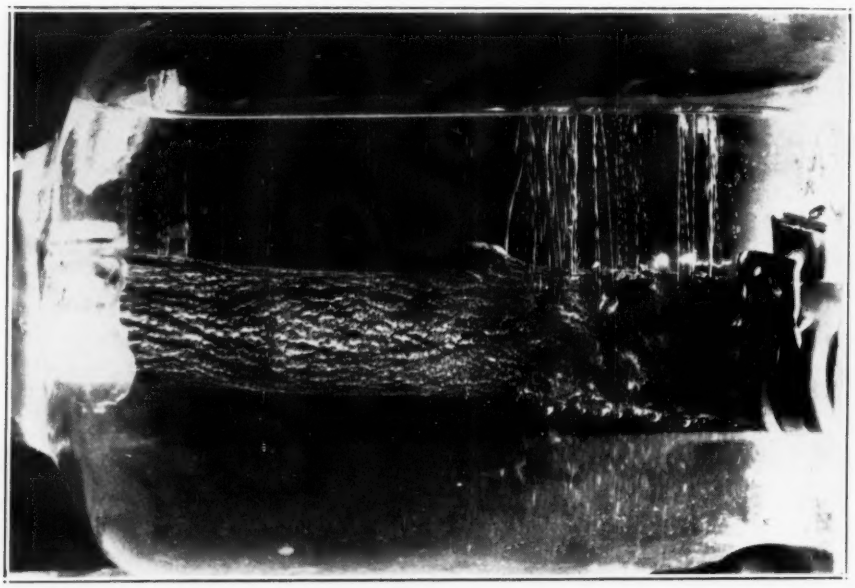

FIG. 8. Section of a citrus branch, with a psorosis bark lesion completely girdling one of its extremities. The affected end of the branch (right) is sealed with an airtight rubber cap; the unaffected end (left) is sealed into one end of a glass tube, which extends through the rubber stopper of the glass jar. The branch is immersed in 95 per cent ethyl alcohol. Suction in the air space above the alcohol was maintained equivalent to 60 cm. of mercury (about four-fifths of an atmosphere). Streams of air bubbles are seen arising chiefly from the psorosis-affected bark and escaping at the surface of the alcohol. $\times \frac{5}{8}$.

tube, which was passed through the rubber stopper of the glass jar. One cut end was thus exposed to the outside air. The branch was placed in the jar, and the stopper was adjusted. The jar had been partly filled with 95 per cent ethyl alcohol and was maintained in a horizontal position during the experiment (Fig. 8). Through a bent glass tube also passing through the rubber stopper, the air was pumped out of the space in the jar above the alcohol so as to maintain a difference in pressure equivalent to 60 cm. of mercury (about four-fifths of an atmosphere).

At first, a great many minute air bubbles moved up from the whole surface of the branch tested, especially if it had partially dried before the

beginning of the experiment. After 5 to 10 minutes, the streams of bubbles could still be seen to proceed from the diseased bark, mainly from the margin of the lesion, while only a few, or none, escaped from the unaffected bark.

In one experiment, for example, only 6 small streams of bubbles were observed to proceed from the unaltered bark, which occupied a length of 6 cm. Because of their great number, it was difficult to count the streams of bubbles arising from the psorosis bark lesion, which occupied a length of about 3 cm., but more than 30 were observed. The bubbles from the diseased portion of the branch were larger than those from the unaffected portion. In the older part of the lesion, occupying 0.5 cm., only 3 or 4 streams of bubbles were apparent. This was probably due to the formation, in this area, of new bark whose permeability was similar to that of unaltered bark.

In figure 8 the streams of bubbles are clearly shown against a black background. Here most of the streams of bubbles may be observed to come from the margin of the lesion between regions of severe scaling and unaffected bark. This is to be expected, since this is the most recently disturbed region of the bark. The experiments were run for several hours without appreciable change in the number or size of the bubbles.

When a young, healthy branch was used in an experiment similar to the one shown in figure 8, using water instead of ethyl alcohol, numerous bubbles were seen to form on the surface of the bark. These bubbles were arranged longitudinally along the lenticels, and apparently no bubbles formed on the portions away from the lenticels. The bubbles remained attached to the point of formation for a considerable period of time and were observed to become detached when they approached a size of about 0.25 mm. in diameter. The time necessary for a bubble to attain this diameter varied considerably. Those forming most rapidly remained attached about 15 minutes. The majority, however, remained attached several hours.

In order to determine which tissues retained most of the air, an experiment similar to the one shown in figure 8 was performed with a healthy branch immersed in water. A circular band, 1 cm. wide, was carefully so scraped around the branch as to remove only the epidermis and the lenticels and leave as much of the cortical parenchyma as possible. On another portion of the branch, the whole bark was removed around the branch and down to the wood for a space approximately 1.5 cm. in width. The branch was then placed in water in a jar, as in the preceding experiment, and suction was applied.

As shown by the air bubbles, considerable amounts of air passed through the bark which had been scraped lightly, while very few air bubbles arose from the band of exposed wood (Fig. 9, A). The bubbles from the scraped bark of the healthy branch were much smaller than those from the unscraped, psorosis-affected bark in the earlier experiment, but the number of streams was greater. From observations of bubbles shown in figure 9, A, it was apparent that most of the air passed from the exposed, cut end of the branch had come in through the wood vessels and then by means of the

medullary rays had passed to its points of escape from the bark. It was, therefore, suspected that the reason why no air passed from the surface of the exposed wood was that during the girdling operation the cambium had been exposed to air and had thus been rendered impervious to air, possibly through clogging of the medullary rays as a result of coagulation of the cytoplasm. The branch, therefore, was taken out of the glass jar, and a new band of bark was removed beyond the already peeled portion, the operation this time being performed under water. When suction was again applied above the surface of the water in which the branch was immersed, a comparatively large amount of air escaped at the point where the wood had

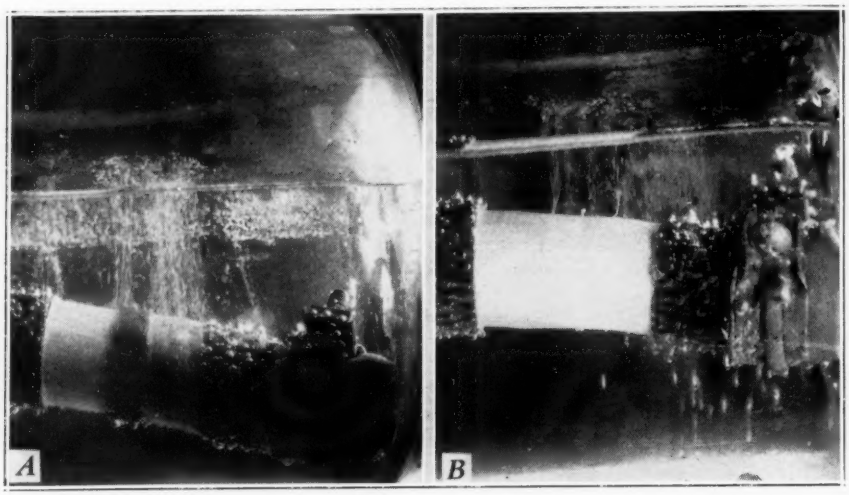

FIG. 9. Air-suction experiment on a section of a healthy citrus branch immersed in water in a glass jar similar to that shown in figure 8. A. Branch girdled by a superficial scraping of the bark (right) and by a peeling away of the bark in the dry air of the laboratory (left). Streams of air bubbles are seen to proceed almost entirely from the scraped area, practically no air being drawn from the exposed wood except at the edges where the vessels were cut. B. The same branch with peeled girdle enlarged by further peeling, under water. Numerous streams of bubbles are now seen to proceed from the newly exposed wood. × about 5/9.

been exposed by removal of the bark under water (Fig. 9, B); thus the hypothesis was confirmed.

As may be seen on the surface of citrus branches, the lenticels form long longitudinal strands of grayish tissue anastomosed laterally and separated by elongated dull-green areas where the epidermis is continuous. The dull-green color is due to the chloroplasts in the underlying parenchyma. In anatomical sections it may be seen that the lenticels correspond exactly to the larger medullary rays, whereas the dull-green areas, still covered with the epidermis, are immediately above the bundles of pericyclic fibers. The lenticels are, therefore, separated from the wood only by the living cells of the bark parenchyma, the medullary rays in the phloem, the cambium, and the medullary rays in the wood. It is obvious that the air passage from the

lenticels to the wood vessels takes place entirely along those tissues of living cells.

By scraping the surface of the bark of a psorosis lesion, the characteristic brown, irregular areas of gummy bark are exposed. If suction is applied to the surface of the water in which a branch thus treated has been immersed, streams of bubbles proceed mostly from the margin of the brown areas. Streams of bubbles of smaller diameter are also seen to proceed from the portions of the lesion that are still green, while none, or very few, come from the brown regions themselves.

In another, similar experiment with a branch 2.5 cm. in diameter and 11.5 cm. long, showing psorosis-B lesions and afterwards found to have a secondary lesion, small streams of air bubbles came from the entire surface of each bark lesion—possibly about the same total amount of air as from equal-sized lesions of psorosis A. A corresponding healthy branch showed less air emerging under the same pressure.

In another series of experiments with wood from a tree infected with psorosis A, disks of bark, 3 cm. in diameter, were sealed to one end of glass tubes and immersed in alcohol over which a suction equivalent to four-fifths of an atmosphere was maintained, as in the previous experiments. No air bubbles were observed in short-time tests (about 5 minutes) with 3 specimens of unaffected bark taken not far from lesions of psorosis. In a similar test with 3 specimens of affected bark from lesions, 0, 2, and 3 streams of bubbles were counted, respectively. The bubbles were approximately 0.2 cm. in diameter. In these tests, in which the bark was without attached wood, the air passed directly from the atmosphere through the inner bark and on to the outer bark and to the alcohol in which the specimens were immersed. Comparable results were obtained also in tests with both healthy and diseased bark, using disks 1 cm. (instead of 3 cm.) in diameter.

From the foregoing experiments, it is concluded that (a) bark with psorosis lesions will allow the passage of considerably larger amounts of air than normal bark; that (b) in normal bark the air passes through the lenticels only; that (c) the epidermis between the lenticels constitutes the principal barrier preventing air from passing freely through the bark; and that (d) most of the air passing through the psorosis lesions comes through minute openings largely located on the margin of the lesions, and more especially on the margin of the brown, gummy areas of the lesions.

DISCUSSION AND CONCLUSIONS

It is known that bark scaling and primary-wood lesions, with certain minor alterations of wood, may be present for a long time in psorosis-affected trees without causing a great amount of tree deterioration. Results of the present study indicate that at these earlier stages of the disease, the passage of water through the wood vessels is only partly impeded; and the amount of starch present in the wood cells is about the same as that in the wood cells of healthy trees. This appears to explain the fact that trees may toler-

ate bark and primary wood lesions for some time with slight or imperceptible decline.

In later stages of the disease, however, when the secondary symptoms appear, that is, when discoloration of the interior wood occurs, water cannot pass through this discolored region or through contiguous regions, and can pass only with great difficulty through wood some distance away from the discolored region. The I–KI starch test, as well as microscopic sections, have shown that all the parts impermeable to the passage of water are devoid of starch, and that the wood vessels in these parts are clogged with gum or a gumlike material.[6] Even in such cases, however, limited regions of wood at various places near the outside of the tree, next to the bark, will be found to conduct water fairly readily. Such areas correspond to regions showing starch reactions with I–KI. It appears that the gradual extension of the regions in the wood in which water passage is blocked may account for the gradual deterioration of the trees.

It was first thought that perhaps the initiation of the discoloration of the wood accompanying the old bark lesions might be related to the passage of air and other gases in and out of the wood by way of the bark. Experiments showed that, under pressure, air passed more easily through the bark of the regions occupied by bark lesions, especially at the margins, than through normal bark. It is therefore concluded that lack of air is probably not responsible for initiation of the secondary lesion. Whether or not increased air has any relation to wood discoloration needs further investigation.

Although there appears to be a close relationship between the extent of the discolored wood and the decrease in water passage, it is possible that still other factors may play some part in the deterioration of the tree. Altered nutrition or toxic substances produced by the discolored-wood region may cause some injury to psorosis-affected citrus trees, but so far, no evidence has been obtained that such factors are important in this connection. That toxic substances are not an important factor is indicated by the observation that only the tree parts above and directly connected with the portion bearing the secondary lesion show deterioration. It would be expected that if toxic substances were present, they would be transported to other parts of the tree and cause injury.

SUMMARY

Two kinds of wood lesions accompany and follow the bark lesions of the two varieties of psorosis virus, psorosis A and psorosis B, on citrus: (a) primary lesions, immediately under the bark lesions, in which gum is formed between outer layers of either normal or altered wood; and (b) secondary lesions, in which discoloration appears to begin in older wood farther inward, and in which starch disappears and the vessels become plugged with gum.

Primary lesions were found to retard water passage only partially.

[6] Tyloses were also looked for but were not noted. Water appears to pass, to some extent, through old, healthy citrus wood.

Their presence usually did not prevent almost normal development of foliage and fruit. In the discolored region of the secondary lesions, however, and in a starch-free region contiguous to the visibly discolored region, the passage of water was completely stopped. Both of these regions were found to be devoid of starch, as indicated by the I–KI test for starch.

The progressive degree of stoppage of water as the secondary lesions increase in extent and lessen the amount of normal water-conducting wood, is considered the main factor in the deterioration of trees affected with psorosis. Impaired nutrition or toxins may also play a small part.

In experiments with air, under a difference in pressure of approximately four-fifths of an atmosphere, it was found that air passed only slowly through normal bark, and only in the regions of the lenticels. Much more air passed at the bark lesions, especially near their margins, than at areas contiguous to these lesions. This indicated that the wood discoloration and the accompanying deterioration of the tree were not caused by lack of air passage at the bark lesions. Lack of water conduction, rather than lack of air passage, appears to be the principal factor in tree deterioration from psorosis.

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POPULATION TRENDS OF PHYSIOLOGIC RACES OF PUCCINIA GRAMINIS TRITICI IN THE UNITED STATES FOR THE PERIOD 1930 TO 1941[1]

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From studies of the annual prevalence and distribution of physiologic races of *Puccinia graminis tritici* in the United States for more than 20 years, it is possible to draw certain conclusions regarding population shifts. Preliminary results have been published (9, 10, 13, 14, 19, 20, 21, 22), and many general statements based on these results emphasize the fact that a variety of wheat may be resistant in one region and susceptible in another in the same year, or that a variety may behave differently in the same place in different years because of differences in the prevalance and distribution of physiologic races.

There have been such decided changes in prevalence of certain physiologic races in the United States since 1930 that it seems desirable to give the data for nine common races for 1930 to 1941, inclusive (Fig. 1). These data are presented to illustrate certain principles, as a summary of all available data would far transcend the limits of a short paper.

SCOPE OF WORK AND METHODS USED

Attempt was made each year to obtain a random and adequate sample of rusted wheat from the entire United States, and some collections of rusted barley and wild grasses also were obtained. The number of collections for each year follows: 1930, 288; 1931, 361; 1932, 325; 1933, 294; 1934, 478; 1935, 787; 1936, 619; 1937, 1,025; 1938, 1,030; 1939, 735; 1940, 902; 1941, 804.

In general, collections were made from a random sample of fields, regard-

[1] Cooperative investigations between the United States Department of Agriculture and the Minnesota Agricultural Experiment Station. Paper No. 2066, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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The writers are indebted to M. N. Levine and Ralph U. Cotter for assistance in determination of physiologic races at various times, and to Donald G. Fletcher for furnishing a large number of collections. Thanks are also due many other individuals for sending collections from time to time; unfortunately the list would be too long if all names were enumerated. Special thanks, however, are due various members of the Bureau of Plant Industry, U. S. Department of Agriculture, and to the personnel of the barberry eradication project of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine. The encouragement and advice of Dr. H. B. Humphrey during the early years of the investigation are gratefully acknowledged.

less of the variety of wheat. For example, when rust was generally prevalent, a collection was made about every 20 miles along the roads being traveled. In some cases, however, special attempt was made to determine

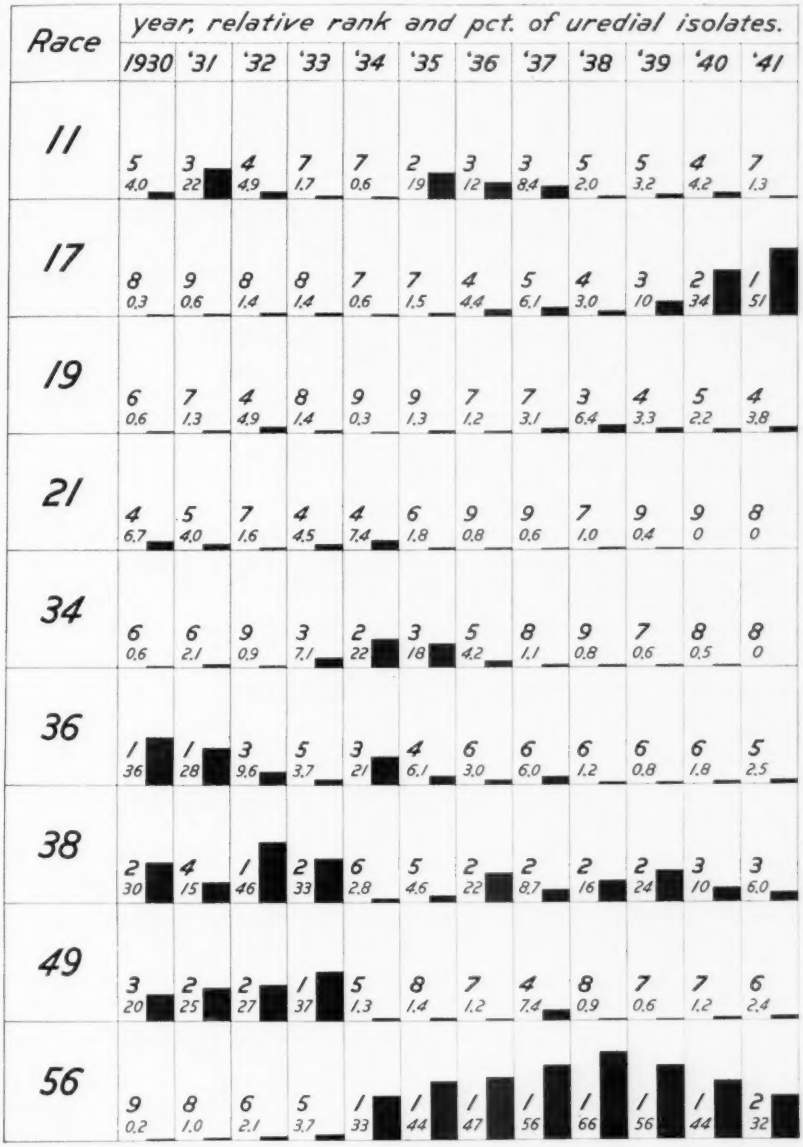

FIG. 1. Population trends of 9 physiologic races of *Puccinia graminis tritici* for the period 1930–1941, inclusive. Lower figure in each block indicates percentage of uredial isolates; upper figure, the relative rank in prevalence.

which races occurred on certain varieties, and it was necessary to depend upon volunteer cooperators for the rust collections in some areas; consequently there was some deviation from random sampling. Moreover, the

largest number of collections always was made in the wheat-growing area extending from Texas northward into the hard red spring wheat region and eastward into the soft red winter wheat area. Relatively few collections were obtained from the Pacific Coast and from the intermountain wheat-growing area of Washington and Oregon. The results, therefore, indicate the general prevalence of races for the country other than the wheat-growing regions west of the Rocky Mountains.

The percentage prevalance figures are an indication of the relative importance of races each year, although the distribution of races was not always completely uniform. Race 38, for example, was more prevalent in Indiana and eastward in some years than elsewhere; and races 56 and 17 were relatively scarce in those areas or localities where varieties resistant to them predominated. With exceptions such as these, there is abundant evidence that the percentage figures given in figure 1 indicate the relative importance of races by years for the country as a whole.

The collections were sent to the federal rust laboratory at University Farm, St. Paul, Minnesota, where races were identified by the methods described by Stakman and Levine (16).

In figure 1 are given the percentages of uredial isolates for each of 9 races for each of 12 successive years. The word "isolate" designates a race identified from a given collection, and its use is necessitated because more than one race often is isolated from a single collection. For example, races 56 and 11 commonly occurred in the same collections in 1935 (Fig. 2). Race 56 produced minute uredia and race 11 large ones on certain durum varieties; therefore, it was possible to identify the races definitely by making appropriate inoculations from the two kinds of uredia. The race obtained from each kind of uredia is an isolate, and in this case two isolates were obtained from one collection. Similarly, there often are two infection types, 2 and 4, on Marquis wheat. By making transfers to certain differential wheat varieties from type-2 uredia and from type-4 uredia, the races can be identified; and each is designated as an isolate. This is explained more fully and illustrated in a report for 1939 (19). Accordingly, the percentages given in figure 1 are based on the total number of times a given race was identified (isolated) in proportion to the total number of times all races were identified; they do not represent the percentage of collections from which each race was identified.

The prevalence of races expressed in terms of percentage of total isolates is, of course, only a relative indication of the situation. The percentage of collections from which each race was isolated may give a better idea of the geographic distribution. The following example is given by Stakman and Loegering (19) in a summary of the results of the survey in 1939:

> Consider race 56. . . . It was identified 590 times, and a total of 1,063 identifications were made of 14 races in 735 collections; that is, there were 1,063 isolates from 735 collections. Accordingly, race 56 constituted 55.5 per cent of all isolates. However, it should be remembered that the percentage figures . . . might be computed on the basis of number of field collections rather than number of isolates. For example, race 56 was isolated from 590 collections of stem rust out of a total of 735 collections, or from 80 per cent of the collections.

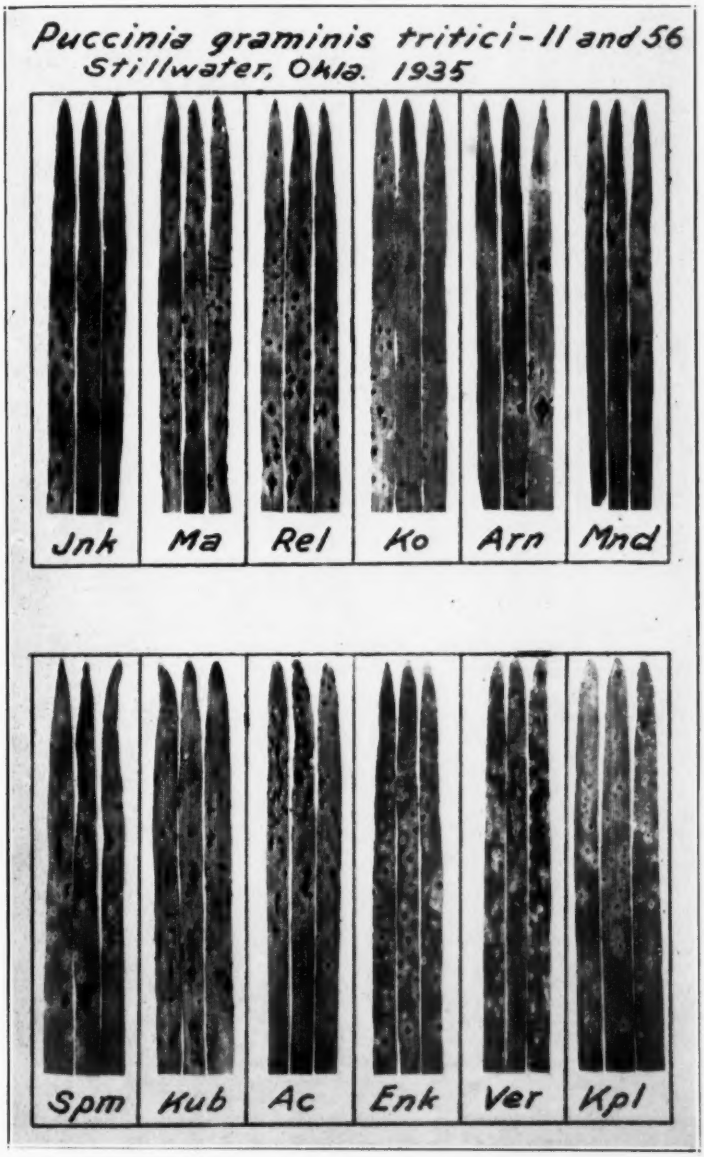

FIG. 2. Differential varieties of *Triticum* spp. inoculated with a collection of rust from Stillwater, Oklahoma, in 1935. This was typical of the infection types produced by many collections of rust in that year. The infection is caused by races 56 and 11. No distinction can be made between them on the first four varieties or on Kubanka, Acme, Vernal, and Khapli. On Arnautka, Mindum, Spelmar, and Einkorn, on the other hand, race 56 causes flecks or type–1 pustules, while race 11 produces type 3–4. There are about a dozen large uredia (caused by race 11) on Arnautka, Mindum, Spelmar, and Einkorn, but there are about 50 flecks or type–1 pustules (caused by race 56) on each of these varieties—all of which may not show in the reproduction. Obviously, therefore, in the original collection, inoculum of races 11 and 56 was present in an approximate ratio of 25: 100. Accordingly, each race comprises 50 per cent of the isolates, but race 56 is by far the more abundant.

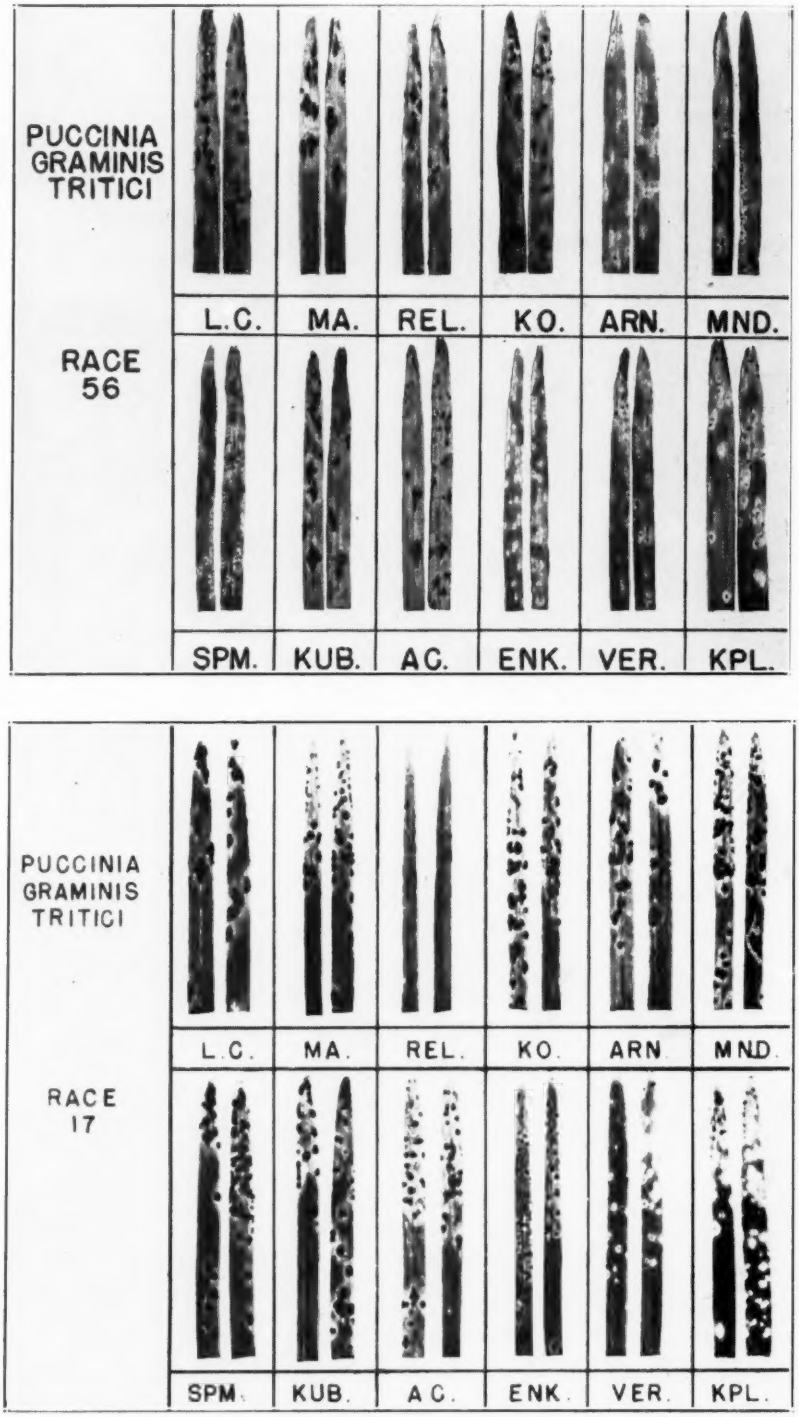

FIG. 3. Differential varieties of *Triticum* spp. inoculated with *Puccinia graminis tritici*, races 56 and 17. Note differences in infection types. Reliance (substitute for

But neither of these percentage figures is entirely adequate because the relative amounts of rust of different races in each collection also are necessary in estimating the importance of each race in producing infection in the field. Results obtained in 1941 furnish a good example (21). Races 17 and 56 were found together in many collections. The infection types produced by these races on the differential wheat varieties are illustrated in figure 3 and are given in table 1.

TABLE 1.—*Infection types produced by races 56 and 17 on differential varieties of Triticum spp.*

Race	LC[a]	Ma	Krd	Ko	Arn*	Mnd*	Spm*	Kub*	Ac*	Enk	Ver	Kpl
56	4	4	4	4	1	1	1	3+	3+	1	1	1
17	4	4	0	3+	4	4	4	4	4	3	1	1

* Durum wheats.

[a] The names of the varieties follow in the order given above: Little Club, Marquis, Kanred (Reliance often substituted), Kota, Arnautka, Mindum, Spelmar, Kubanka, Acme, Einkorn, Vernal, Khapli.

Type 0 indicates immunity; type 1, very resistant; type 3, moderately susceptible; type 4, very susceptible.

Kanred is susceptible to race 56 and immune from race 17, but Einkorn and three of the durums, Arnautka, Mindum, and Spelmar, are resistant to 56 and susceptible to 17. As attempt is made, except for special purposes, to obtain a random sample of rust in the fields from which collections were made and to inoculate the differential varieties with a random sample of the rust in the collection, the infection types characteristic of each race should appear on the differentials in about the same ratio as that between the numbers of spores of each race in the collection. In many collections made in 1941 the ratio of race 17 to race 56 was 85:15, 90:10, or even 95:5. For example, a 90:10 ratio is arrived at as follows:[3] Assume that there are 10 uredia on each leaf of those varieties susceptible to both races, but only one uredium on Kanred. On Einkorn and the 3 durums, Arnautka, Mindum, and Spelmar, on the other hand, there are 9 large uredia and a single minute uredium or fleck on each leaf. If transfers are made from the rust on Kanred or from the minute uredia on Einkorn or the 3 durums, race 56 will be obtained; and from the large uredia on Einkorn or on the 3 durums, race 17 will be obtained. If this is the average ratio for many collections, then each race occurs in 100 per cent of the collections and each comprises 50 per cent of the isolates. But the number of spores, potential inoculum in the field, for races 17 and 56 is in the ratio of 90:10. Clearly, then, race 17 would be the more important. In reality, however, there usually is fairly close agreement between percentage of collections, percentage of isolates, and actual amount of rust, especially for predominant races.

Kanred) is susceptible to race 56 and immune from race 17, while Arnautka, Mindum, Spelmar, and Einkorn are highly resistant (almost immune in the field) to race 56 and susceptible to race 17. (See table 1 for names of differentials.)

[3] A description and diagram of the method of estimating these ratios is given in the report of the race survey for 1939 (19).

For example, in certain years race 56 might almost have been the only race present for practical purposes, as it ranked first in prevalence by a wide margin, by both methods of computation, and constituted 85 to 90 per cent of the rust inoculum in many regions of the United States (12). Nevertheless, it would be desirable, if time and facilities permitted, to make computations designed to express the relative importance of races in terms of amount and distribution of inoculum.

These facts have an important bearing on the number of replications necessary to testing wheat varieties for rust resistance in the absence of artificially induced epidemics in which known races are used. Clearly, the amount of inoculum of the various races in given years and regions is a basic factor in determining whether the varieties have been adequately tested, assuming that weather conditions were favorable for rust development.

RESULTS

Several facts become immediately apparent on inspecting figure 1. Some races were notably prevalent early in the 12-year period and then declined; others have increased from small beginnings; others have fluctuated considerably; and one, race 34, increased gradually in prevalence, and then decreased just about as gradually.

Race 56 has had the most spectacular career. A brief statement regarding this race was published in 1938 (9). It was first identified in the United States in 1928 and increased slowly in prevalence for 6 years, then suddenly assumed first rank in 1934 and continued to increase until it reached its peak in 1938. In 1939 it began to decline and continued to do so through 1940 and 1941; nevertheless, for 7 successive years, 1934 to 1940, inclusive, this race occupied first rank, usually by a wide margin. How long the decline, which began in 1939, will continue is a question, but the drop to 32 per cent in 3 years from the high of 66 per cent in 1938 certainly indicates a definite downward trend.

Races 36 and 49 are in direct contrast with race 56: they declined as race 56 increased. Race 36 was the most prevalent of all races in 1930 and 1931, declined in the next 2 years, then increased again in 1934, but since that time it has not been sufficiently prevalent to be really important. Race 49 increased for the first four years of the decade beginning with 1930 until it attained first rank in 1933; in 1934 it decreased sharply, and since that time it has been relatively rare except in 1937.

Races 11 and 38 have fluctuated considerably, without displaying definite trends.

Race 34 comprised 0.6 per cent of all isolates in 1930, increased until it attained second rank in 1934, with 22 per cent of all isolates; but since then it declined each year and was not isolated at all from uredial material in 1941.

The history of race 17 is significant. It was common in the first half of the decade 1920 to 1930, then decreased until it reached a low of 0.3 per

cent of all isolates in 1930 [unpublished data and Wallace (24)], after which it tended to increase slowly and somewhat irregularly. During the last 3 years it increased sharply and surpassed race 56 in 1941, attaining first rank, with 51 per cent of all isolates.

Races 17 and 56 together comprised most of the inoculum of stem rust in 1940 and 1941, making up 78 per cent and 83 per cent of all isolates in the two years, respectively. In both years race 38 occupied third rank in prevalence. In those years these 3 races, with 88 and 89 per cent, respectively, of all isolates, were the races to which wheats in general were exposed. No other races were sufficiently prevalent to be important practically, except in local areas. This illustrates the present tendency for a few races to predominate among the uredial isolates. This tendency is not nearly so pronounced, however, among aecial isolates, as shown by the data for 1940, which are given as an example.

A comparison of the data for uredial isolates and those for aecial isolates in 1940 is given in figures 4 and 5. Races 56, 17, and 38 occupied first, second, and third positions, respectively, among both groups of isolates. Race 36, however, was obtained much more frequently from aecial collections than from uredial collections; and this is true of race 34 also, which at one time had been very prevalent on wheat but which comprised only 0.5 per cent of all uredial isolates in 1940. It will be noticed that race 15, the most generally virulent race found in the United States, comprised 5 per cent of the aecial isolates in 1940, but was obtained in only 1 per cent of the uredial isolates.

The importance of barberries in producing and perpetuating races previously has been discussed by Stakman, Levine, Cotter, and Hines (17), and has been summarized by Stakman, Loegering, and Cotter, (22) in the report of the physiologic race survey for 1940 as follows:

> Races 9, 10, 14, 24, 40, 55, 69, 77, 79, 83, 117, 121, 126, 140, 146, and 147 of *Puccinia graminis tritici* were isolated only from barberries or from rusted wheat in the area where barberries become rusted. Barberries definitely were responsible for the occurrence of these races in 1940, as they were not found at all in Mexico, Texas, or Oklahoma, from which urediospores can be disseminated in the spring. Regardless of the relative amounts of rust resulting from wind-blown urediospores from the far south and from aeciospores produced on barberries, it is apparent that more kinds of rust races come from barberries. Race 56 was known in the barberry area for at least five years before it appeared in Texas and became established in northern Mexico. The predominance attained by this race caused farmers to discard Ceres wheat, and there is the possibility of a similar history in the future with respect to some of the rarer rust races and some of the newer varieties of wheat.

The practical importance of population shifts in physiologic races has been alluded to (10, 18, 19, 20, 21, 22). It is worth emphasizing, however, that Ceres wheat, grown commonly in the spring wheat region after its distribution in 1926, is so susceptible to race 56 that it was virtually ruined by rust in 1935, the first favorable rust year after this race had become abundant, and it was again severely injured in 1937. Stakman and Cassell (9), Johnson and Newton (7), and Stakman, Loegering, and Cotter (22) have summarized evidence regarding the role of race 56 in eliminating Ceres

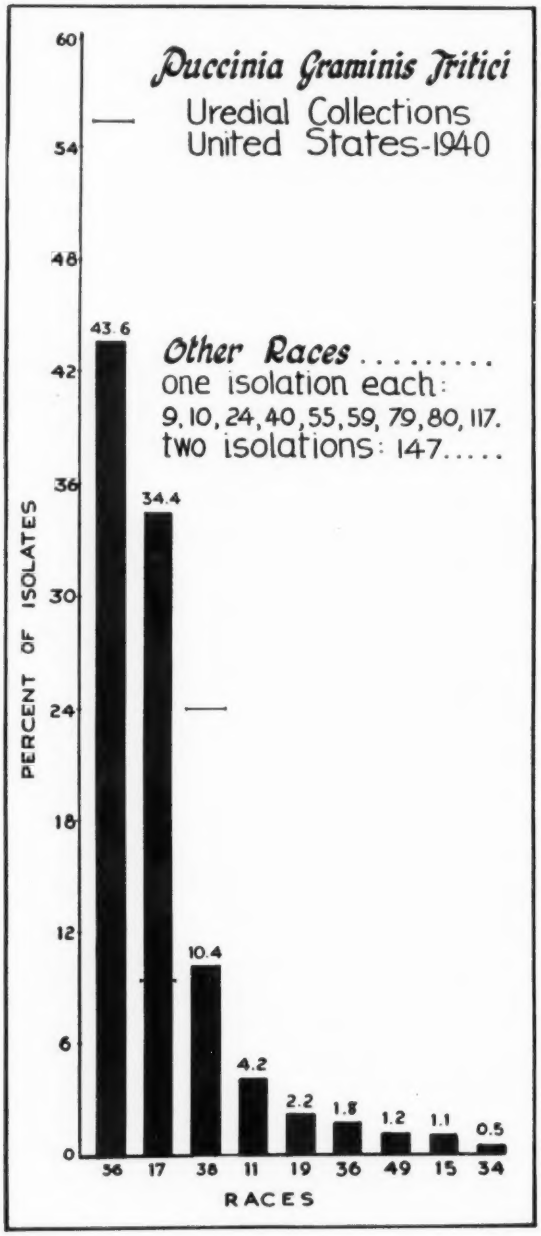

FIG. 4. Percentage of isolates of physiologic races of *Puccinia graminis tritici* obtained from uredial collections in 1940.

wheat. There is no question but that race 56 is more virulent than many other races on Ceres (Fig. 6). It often is possible to separate it from other races growing on seedling plants of Kota or Ceres in the greenhouse by simply making transfers from the largest pustules. So conspicuous is its

virulence on Ceres that an experienced observer can make a rather accurate guess as to its identity on differential hosts in the greenhouse merely by observing the size of the uredia on Ceres. Moreover, it seems particularly able to thrive on this variety at temperatures higher than those at which certain other races will thrive[4] (1). The tremendous increase in acreage of Ceres and a succession of unusually hot summers apparently favored the rapid increase of this race. Several other races might have eliminated Ceres had they become as prevalent as race 56; but the record shows race 56 responsible.

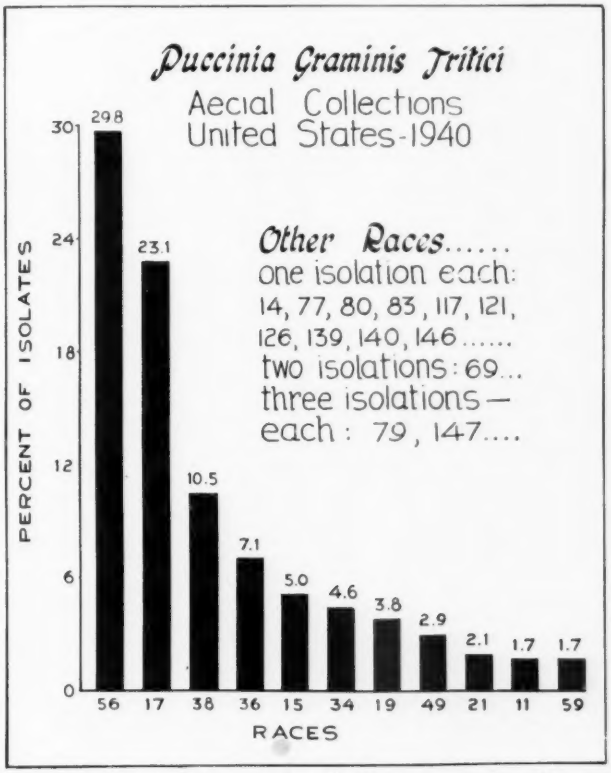

FIG. 5. Percentage of isolates of physiologic races of *Puccinia graminis tritici* obtained from aecial collections in 1940.

Thatcher wheat, first distributed in 1934, has very largely supplanted Ceres in the spring wheat area. It withstood the epidemic of 1935 so well that the acreage was increased greatly the next year. This variety is the result of a double cross (Marquis × Iumillo) × (Marquis × Kanred). It has the immunity of Kanred from a number of physiologic races and has considerable adult-plant resistance derived from Iumillo durum (3). Thatcher has not had a severe rust test under natural conditions, however, because it is highly resistant to or immune from those races that have been most

[4] Cassell, Robert C. Factors affecting the distribution of physiologic races of *Puccinia graminis tritici* Erikss. and Henn. University of Minnesota Ph.D. thesis. 1938.

prevalent since its introduction. Thatcher is moderately resistant in the seedling stage and highly resistant in the adult stage to race 56; and it is immune in all stages of development from races 17, 19, 21, and 49. Consequently, there has been a relatively small population of physiologic races to which it is susceptible. With the present predominance of races 17 and 56, Thatcher is still safe from serious damage. Nevertheless, it is susceptible to several races, including 36 and 15B, and has been heavily rusted in field plots.[5] If these races or still others to which Thatcher is susceptible become

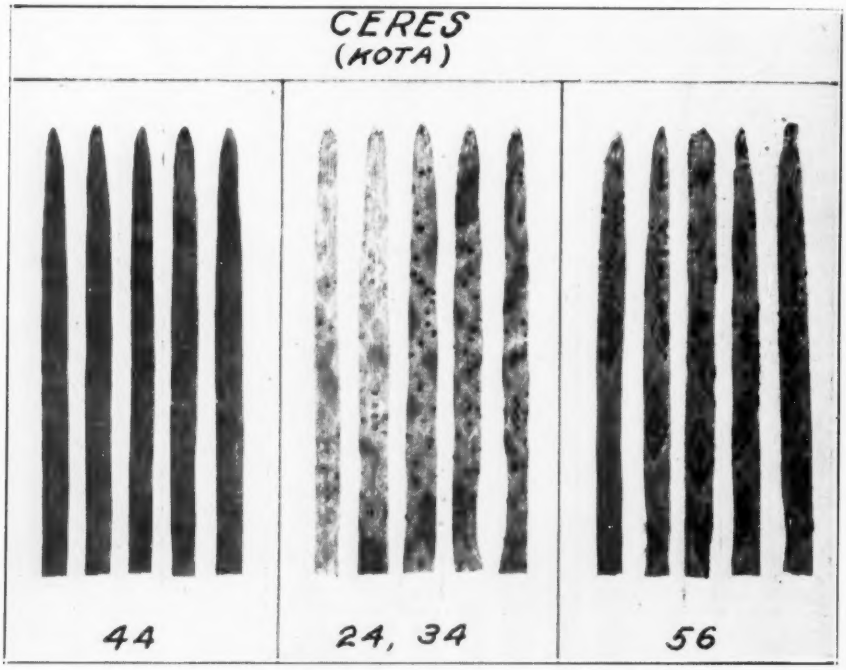

FIG. 6. Ceres wheat inoculated with 4 races of *Puccinia graminis tritici*, showing immunity from race 44, resistance to race 24 (two plants), moderate susceptibility to race 34, and extreme susceptibility to race 56.

prevalent and abundant, it is likely that it may be injured by rust when weather conditions favor rust development.

Race 17 was by far the most common race in much of the hard red winter wheat region in 1941. The behavior of wheat varieties was exactly what one would expect from the results of the physiologic-race survey. Tenmarq and other Kanred derivatives were relatively rust-free, whereas many of the soft wheats in neighboring fields were severely rusted. Similar observations were made in the spring wheat region. Thatcher was virtually rust-

[5] Thatcher was heavily rusted in replicated plots in the Cooperative Rust Nursery at St. Paul, Minn., in 1936. Race 36 was commonly isolated and seemed to be primarily responsible for the rust. Thatcher also has been heavily rusted in several recent years in field-plot experiments made by Helen Hart at St. Paul, the results of which have not yet been published.

free, whereas Marquis and Ceres, both susceptible to races 17 and 56, rusted rather heavily where conditions were favorable. Moreover, there was more rust on Mindum durum than on Thatcher.

The situation with respect to the durums, therefore, has fluctuated. Several years ago, when races 11, 21, 34, and 38 were prevalent, the most commonly grown durum varieties, especially Mindum, were severely rusted (15). During the past few years, however, when race 56 predominated, there was very little rust on the durums, except Kubanka, because most of those commonly grown are almost immune from this race. With the increase in race 17, however, they are again in jeopardy.

Although most varieties of wheat now grown may be injured more or less severely by certain races of rust, it must be emphasized that many of the varieties now available, especially in the hard red spring wheat group, are very much less liable to damage than the varieties that they replaced. Remarkable progress has been made in developing varieties with physiologic resistance to many races and with a certain degree of adult-plant resistance, due to morphologic and other characters, to most of the races. These varieties are, therefore, likely to remain resistant in more localities and in more seasons than those developed before there was sufficient recognition of the necessity for testing against many rust races under a wide range of environmental conditions in the breeding program.

Unfortunately, little is known definitely about the factors affecting changes in populations of physiologic races. It has been shown by several investigators[6] (1, 2, 4, 5, 6, 8) that environmental factors affect different races differently. Some of them develop best on certain varieties of grain at relatively high temperatures; others at low temperatures. The final amount of rust that develops on wheat depends on the physiologic race of rust, the variety of wheat, the effect of environment on each, and its effect on the interaction between the two. It seems likely, therefore, that seasonal temperatures may play an important part in determining prevalence of races. The prevalence of certain wheat varieties obviously has an effect also. Kanred, for example, which is immune from a number of races, would tend to decrease their prevalence over a considerable area. Likewise, if Mindum and similar durum varieties were grown exclusively in North Dakota, it would be quite impossible for race 56 to become prevalent there; and as long as Thatcher is grown in the spring wheat area there will be a tendency for it to reduce the prevalence of races 56 and 17 in that area and, consequently, the percentage prevalence in general.

Factors that affect the overwintering of various races in the uredial stage in northern Mexico and central Texas, the area in which uredial overwintering is most likely to occur, undoubtedly are important when considered in connection with the factors affecting the subsequent development of a large amount of inoculum in those areas and the widespread dissemination of spores northward. Fortuitous circumstances probably affect this

[6] See footnote 4.

situation somewhat, but there is evidence that certain races can overwinter more easily than others. Thus some may tend to be eliminated during the winter while others survive. Factors affecting the spread of the rust from these areas, therefore, would be important in determining the prevalence of the overwintered races in the country as a whole (23). Moreover, meteorological conditions affecting the dissemination of rust from areas where barberries become heavily rusted would also be important, as they appeared to be in 1942 (11). Although detailed results are not given for 1942, it is clear that a decided increase in prevalence of race 38 was due to the fact that it was produced commonly on barberries in the Virginias and Pennsylvania. Conditions were favorable for rust development in those areas, and for subsequent dissemination of inoculum westward into Ohio, Indiana, part of Illinois, and southern Michigan. While the prevalence of race 38 was rather high for the country as a whole, therefore, its prevalence was far higher in the States just mentioned than elsewhere.

SUMMARY

Annual physiologic-race surveys of *Puccinia graminis tritici* have been made in the United States for more than 20 years. Results for 12 years are summarized in this paper, as they illustrate the decided shifts that may occur in the prevalence and importance of races.

During the 12-year period 1930–1941 some races have increased greatly, others have decreased, some have fluctuated irregularly, and one increased during the first half of the period and then decreased at about the same rate.

During the same period 5 races have ranked first in prevalence and amount in one or more years, namely, races 36, 38, 49, 56, and 17.

During the first half of the 1930–1939 decade races 36, 38, and 49 were the most important for practical purposes. During the last half of the decade race 56 was by far the most important, ranking first in prevalence for 7 successive years. Since 1939 races 56, 17, and 38 have been by far the most important and prevalent.

The meteoric rise of race 56, followed by a tendency to decline, and the decided increase of race 17 in 1940 and 1941 are especially significant. At present these two races appear to constitute at least 90 per cent of the inoculum in the Mississippi Basin.

Because of the spectacular rise in prevalence of race 56, beginning in 1934, Ceres wheat is no longer classed as resistant. With changes in the prevalence of races, it is possible, however, that Ceres might again attain to the resistant class.

During the past several years race 56 alone or in combination with race 17 has comprised between 80 and 90 per cent of the rust inoculum, at least in the spring wheat area. Thatcher, which has largely replaced Ceres, is resistant to race 56 and immune from race 17; therefore Thatcher has not yet been exposed to abundant infection, under favorable conditions, by a race or combination of races to which it is susceptible.

The population shifts of physiologic races show the practical need for extensive replication in time and space in testing varieties for stem rust resistance, unless the varieties are exposed to artificially induced epidemics created by inoculating with all races that occur or are likely to occur in the region for which the variety is intended.

Comparison of the number and kinds of races isolated from or near barberry bushes with those isolated away from barberries shows that the eradication of the alternate host as completely as possible is of paramount importance in reducing the number of races and preventing the production of new ones. Details for 1940, taken as a sample year, are given in the text.

The changes in relative proportions of individual races in the total population may be gradual or sudden; accordingly, the susceptibility and resistance of wheat varieties in the field tend to vary directly with these changes, although this tendency may be affected considerably by the additive effects of several races and by conditions that are favorable or unfavorable for rust development.

There is relatively little definite information regarding the causes for changes in prevalence of races, although seasonal temperatures, distribution of wheat varieties, conditions affecting the winter survival and subsequent development and dissemination of inoculum of different races from the far South, and the relative amounts of different races produced on barberries and disseminated from them all appear to be important.

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A COMPARISON OF LABORATORY AND FIELD RETENTION AND PROTECTIVE VALUE OF CERTAIN COPPER FUNGICIDES[1]

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The need for reliable methods of testing fungicides is recognized by almost every worker in the field of plant pathology. The large amount of published data on this subject testifies to the demand for information on the fungicidal properties of materials before they are actually used in the field. The development of many new compounds, particularly organics and substitute copper materials to replace Bordeaux mixture, has stimulated the demand for methods that would more accurately evaluate the properties influencing the field performance of a fungicide.

Millardet and David (23), Girard (6), and Perraud (24) suggested very early that retention[3] might be an important factor influencing the protective value of copper fungicides. Kadow (14), Daines (2), Marsais and Segal (19), Hamilton (9), Martin (22) and Fajans (3) also have pointed out the importance of the retention factor in the performance of this type of fungicide. The early work of Guillon and Gouirand (8) on laboratory tests of retention also involved a correlation of this type of data with that obtained in the field. Quite comparable results were found on grape leaves and glass plates, where both surfaces were subjected to artificial rain and the amount of copper remaining was determined by chemical analysis. Kehlhofer (15) also reported some agreement between retention on glass plates and grape leaves as determined by chemical analysis. A good correlation between retention of certain copper fungicides, as determined by spore germination tests and by chemical analyses, was found by McCallan and Wilcoxon (16). Laboratory studies of retention of copper fungicides also have been reported by Heuberger (11, 12) and Young and Beckenbach (28), and field studies using chemical analysis by Magie and Horsfall (18) Hartmann (10) and Green and Goldsworthy (7).

The studies here reported involved the determination of the retention of certain copper spray materials by (a) direct chemical analysis before and after artificial rain on Pyralin plates and (b) chemical analysis of leaves before and after weathering in the field. The results of (a) and (b) were correlated with a third measure of retention (c), as determined by spore

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[3] The term "retention" is used here as defined by the Committee on Standardization of Fungicidal Tests (1) in preference to "tenacity" or "adherence."

germination tests very kindly conducted by Doctor McCallan. Correlation of these measures of retentions with control data was determined for 1941. Toxicity determinations furnished by McCallan also were compared with this control data.

METHODS

The method of laboratory spraying of cellulose nitrate (Pyralin) plates by atomizing on a rotating turntable has been described and illustrated by Worthley and Frear (27). For the retention studies an unwashed lot of 5 sprayed plates was designated as the original. Five were removed after being washed by atomizing with 0.25 l. of distilled water, 5 more after atomizing with an additional 0.5 l. of water, and the final lot of 5 after with 1.0 l. more of water. Preliminary work indicated that an interval of 2 weeks was necessary between spraying and the washing of the plates with water. The total amount of water reaching each plate in all 3 washings was approximately 0.485 inches.

The spore germination tests of the 1940 and 1941 materials as determined by S. E. A. McCallan involved the use of a settling tower (McCallan and Wilcoxon (17)) for spraying glass plates, and the determination of LD50 values (Wilcoxon and McCallan (26)) for *Sclerotina fructicola* on unwashed slides and on those subjected to an inch of laboratory rain delivered through a rose-type nozzle (McCallan and Wilcoxon (16)). From the ratio LD50 weathered slides/LD50 unweathered slides the tenacity index was obtained.

The field spraying was done with a commercial-type orchard sprayer on cherry trees not more than 15 years of age. Single-tree randomized plots replicated at least 6 times were always employed. After certain preliminary work it was found necessary to take approximately 180 leaves per plot for the method of analysis used prior to 1941 and 50 per plot in that year. Sampling in 1940 and 1941 was confined to fruit spur leaves on cherries (Montmorency).

The area of leaves was determined photoelectrically after the method of Frear (4). Since the use of alternating current introduced an error due to fluctuation in the original reading, an apparatus was built in 1940 of the same type but using storage batteries with a current adjustment that always gave the same amount of light. Copper analyses were expressed as milligrams per square meter (both plates and leaves). Leaf-area calculations were based on 2 surfaces per leaf.

Methods of copper analysis on plates and leaves have been described by Frear (5) and involve the use of a photoelectric colorimeter. Prior to 1941 leaves were analyzed for copper by the electrolytical method.

Preliminary studies in 1938 on apple and cherry and, in 1939, on the latter, as well as considerable laboratory work, indicated that certain refinements in technique were necessary for obtaining accurate determinations of retention. Marked differences in retention were found for certain of the copper materials tested, but extreme variability precluded any valid con-

elusions. In view of this, retention studies in the field in 1940 and 1941 were confined to cherry leaves where the copper residue was found to be less variable than the apple fruit and leaf surfaces. Copper analyses were made separately for each of the 6 replicates in order that the data might be subjected to analysis of variance. The spray formula was exactly the same in both laboratory and field.

RESULTS

The kind and amount of materials used for each treatment in both laboratory and field are given in table 1. The tank-mix copper phosphate used in 1940 was prepared by dissolving 2 lb. of copper sulphate directly into the tank and then adding 2 lb. of trisodium (Treatment 7) or disodium (Treatment 22) phosphate. Three pounds of hydrated lime were added last. Instant Bordeaux mixture was prepared following the procedure described by Schneiderhan (25). Lead arsenate was used at the rate of 2 lb. per 100 in the first 3 sprays in 1940 and in the first 2 in 1941.

The standard 4-spray schedule, consisting of petal-fall, shuck-fall, pink-fruit, and post-harvest applications, was supplied in the field. The first sample was taken in the field the same day that the post-harvest spray was made, and the residue found at this time is referred to as the original deposit. This deposit consisted of a weathered residue remaining from previous applications and the unweathered portion from the spray applied immediately before the sampling. The variation in the amount of copper found at this time as given in table 1 is suggested to be a reflection of the varying retention from the previous sprays as well as of the normal variation in field spraying. The laboratory spraying involved only one application with no lead arsenate since some preliminary work indicated that this material did not significantly affect retention of copper on the plates.

To compare the retention of various materials, calculations of percentage copper remaining were made based on this original residue on the leaves and the unwashed plates in the laboratory. These percentages are given in table 1, together with actual amounts of copper in the non-weathered deposit on the leaves and unwashed plates.

In order to facilitate comparisons of the retention on leaves and on Pyralin plates with that obtained by spore germination tests, indices of retention were calculated from the data in table 1 to give a single figure to express the retention of a given material on either the plates or leaves. The percentage of copper remaining on the leaves at a given sampling date was multiplied by the total rainfall before this sampling and a total obtained by adding these 3 values for each of the treatments. The index was calculated by dividing this total by the sum of the rainfall for the 3 weathering periods multiplied by 100 to obtain a value less than 1. Thus the index for treatment no. 1 in 1940 was obtained as follows:

$$\frac{(100.5 \times 3.7) + (72.9 \times 2.8) + (38.2 \times 4.9)}{(3.7 + 2.8 + 4.9) \times 100} = 0.669$$

TABLE 1.—*Original copper deposits and percentages remaining after weathering of leaves and washing of plates*[a]

Treatment[b]	Leaves				Plates			
	Sampling				Amt. water sprayed on plates			
	1st (orig.)	2nd	3rd	4th	None (orig.)	0.25 l.	0.5 l.	1.0 l.
1940	*Mg.*	*Per cent*	*Per cent*	*Per cent*	*Mg.*	*Per cent*	*Per cent*	*Per cent*
1. Bord. 2–4–100[c]	38.7	100.5	72.9	38.2	21.8	99.8	101.5	100.1
7. Tank-mix copper phos.	40.6	91.2	67.5	38.1	20.8	101.6	101.2	100.9
11. Tenn. "26" 3–3–100	36.5	66.1	45.2	16.1	35.2	85.7	63.9	51.8
12. " +1 pt. Orthex	43.6	74.8	57.2	23.3	35.8	90.9	73.8	62.4
13. " +1 pt. Nufilm	40.3	56.4	45.6	11.7	30.1	94.1	81.1	65.1
14. " +1 pt. Spralastic	46.7	73.9	49.1	18.1	35.5	90.2	69.0	53.0
15. " +Summermulsion Spredrite	45.4	79.8	60.8	23.3	35.5	98.2	83.3	70.6
22. Tank-mix copper phos.	44.7	79.6	66.5	29.8	22.0	99.6	100.3	101.6
Least sig. diff. (19:1)	.....	12.1	9.9	7.0	.....	.....	.....	.....
1941								
3. Bord. 2–8–100	38.4	68.1	41.1	30.0	18.0	97.1	88.4	89.9
4. Cupro-K 3–3–100	40.0	50.3	35.5	25.6	20.3	92.6	63.9	50.1
5. " +1 pt. S.E.C. oil	47.5	62.8	42.7	37.2	28.5	90.7	82.0	84.3
6. Tenn. "26" 3–3–100	42.2	41.3	36.3	24.4	30.6	83.1	56.9	44.1
7. " (dolomitic lime)	45.3	43.1	29.8	25.6	33.7	65.7	51.5	40.2
8. " "34" 2½–3–100	40.9	45.4	34.2	31.5	29.2	78.8	72.3	54.6
9. Copper Hydro "40" 3–3–100	53.2	38.4	28.2	30.8	33.9	77.3	52.5	35.6
10. Copper "A" 1½–3–100	33.3	34.5	22.6	24.0	25.1	70.2	46.0	31.5
11. Coposil 3½–3–100	35.0	35.7	28.4	23.5	28.4	74.3	54.5	32.3
12. Bordow 6–3–100	41.7	35.3	32.1	20.6	31.9	75.6	57.5	48.7
15. As 6+1 qt. Spralastic	53.7	43.2	34.0	24.4	35.8	54.2	51.9	37.8
16. As 6+½ lb. soya flour	46.7	33.5	32.7	21.9	27.8	60.3	41.2	36.5
18. Copper "A" 1½–8–100	33.5	34.7	29.5	25.0	28.3	55.5	37.0	23.7
20. Same as 6	48.3	35.6	25.7	22.2		(See Treatment No. 6)		
Least sig. diff. (19:1)	.....	6.3	5.8	4.8	.....	.....	.....	.....

[a] Copper expressed as milligrams per square meter in original deposit and as percentage of the original after the three weathering periods in the field and three washings in the laboratory. Percentage values are an average of the percentage remaining in six blocks in the field and of two tests of five plates each in the laboratory.

[b] Treatment numbers omitted were not sampled.

[c] The first figure in all the treatment formulae represents pounds of copper material and the second, pounds of hydrated spray lime (high calcium lime, except where indicated otherwise).

The index for plates was obtained in the same way with the percentage copper remaining on the plates and amounts of water used in the washing process. These indices are given in table 2. The control for 1941 is expressed as the percentage leaves on October 10 with no leaf spot (*Coccomyces hiemalis*) on 4 tagged branches with 50 leaves per tag originally present. Differences in control in 1940 were not significant and these data are not given. Rainfall for the sampling periods in the field is shown in figure 1.

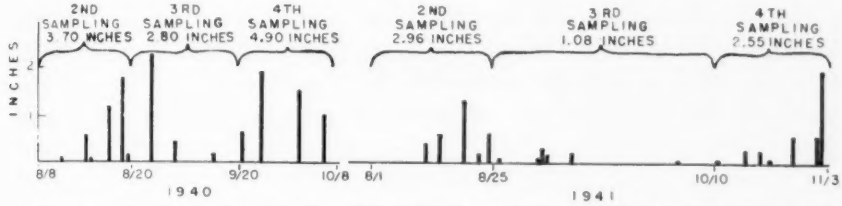

FIG. 1. Sampling dates and distribution of rainfall in 1940 and 1941. Total rainfall for each weathering period also is given.

TABLE 2.—*Summary of data obtained in 1940 and 1941*

Treatment	Retention index[a]		Tenacity index	LD50	Control
	Plates	Leaves			
1940					
1. Bord. 2–4–100	1.000	0.669	0.555	0.190	.....
7. Tank-mix copper phos.	1.010	0.626	0.465	0.230	.....
11. Tenn. "26" 3–3–100	0.605	0.395	0.190	0.350	.....
12. " +1 pt. Orthex	0.698	0.483	0.235	0.360	.....
13. " +1 pt. Nufilm	0.738	0.345	0.170	0.330	.....
14. " +1 pt. Spralastic	0.629	0.438	0.085	0.300	.....
15. " +Summermulsion Spredrite	0.782	0.508	0.070	0.240	.....
22. Tank-mix copper phos.	1.000	0.550	0.410	0.210	.....
1941					
3. Bord. 2–8–100	0.905	0.489	0.570	0.150	64.7
4. Cupro-K 3–3–100	0.602	0.383	0.345	0.440	20.2
5. " +1 pt. S.E.C. oil	0.845	0.495	0.240	0.590	27.7
6. Tenn. "26" 3–3–100	0.531	0.339	0.150	0.460	45.4
7. " (dolomitic lime)	0.471	0.341	0.135	0.320	46.7
8. " "34" 2½–3–100	0.631	0.382	0.310	0.360	54.2
9. Copper Hydro "40" 3–3–100	0.464	0.337	0.305	0.590	42.1
10. Copper "A" 1½–3–100	0.412	0.285	0.185	0.350	41.0
11. Coposil 3½–3–100	0.447	0.298	0.140	0.370	34.3
12. Bordow 6–3–100	0.548	0.291	0.255	0.220	61.7
15. As 6+1 qt. Spralastic	0.442	0.344	0.185	0.440	41.4
16. As 6+½ lb. soya flour	0.413	0.289	0.330	0.470	43.4
18. Copper "A" 1½–8–100	0.321	0.301	0.085	0.300	32.0
20. Same as 6	.....	0.288	.....	.....	.....

[a] Retention index was determined on Pyralin plates in laboratory and cherry leaves in the field. Control is expressed as percentage disease-free leaves. Tenacity index and LD50 were determined by S. E. A. McCallan.

An inspection of table 2 indicates a good correlation between certain of the methods. It is to be noted that in both years the laboratory washing method used on Pyralin plates failed to cause any loss of the Bordeaux mix-

calcium lime, except where indicated otherwise).

ture residue, although the technique used in the spore-germination tests on glass plates did. The same was true for the 2 tank-mix copper phosphate treatments in 1940. These materials also showed a higher retention on the leaves than any of the Tennessee "26" formulae in that year. Summermulsion and Spredrite, Spralastic, and Orthex all acted as stickers in increasing the retention of Tennessee "26" on Pyralin plates and leaves. Nufilm did not increase retention on the leaves nor on the glass plates in the spore-germination tests. Tennessee "34" was higher in retention than Tennessee "26" on all three surfaces and also in control. Copper "A" was low in all the tests, as well as in control, and the additional lime in treatment 18 appeared to have no effect. Tennessee "34" was better than Copper Hydro "40" in all 3 tests. Dolomitic lime with Tennessee "26" did not affect retention or control as compared with treatment 6 using the regular high calcium spray lime. Cottonseed oil emulsion (S.E.C. oil) increased retention of Cupro-K, as well as control. Copper "A" and Coposil were low in retention in all 3 tests, but Bordow was low on the leaves.

In order to compare more accurately the correlations of the data given in table 2, correlation coefficients were calculated (Table 3). There is evi-

TABLE 3.—*Correlation of retention indices on leaves and plates, tenacity indices, LD50 and control as given in table 2*[a]

Factors correlated	Year	Correlation coefficient
Retention indices on Pyralin plates and leaves	1940	0.845
	1941	0.930**
Retention index on leaves, and tenacity coefficient	1940	0.809**
	1941	0.584*
Retention index on Pyralin plates, and tenacity coefficient	1940	0.856**
	1941	0.679**
Control and retention index on leaves	1941	0.034
Control and retention index on Pyralin plates	1941	0.247
Control and tenacity coefficient	1941	0.413
Control and LD50[b]	1941	−0.614*
Control, retention index on leaves, and LD50	1941	0.617
Control, retention index on plates, and LD50	1941	0.641
Control, tenacity coefficient, and LD50	1941	0.679*

* Significant at odds 19: 1.
** Significant at odds 99: 1.
[a] Plot 20, in 1941, omitted.
[b] Negative coefficient, because low LD50 indicates high toxicity.

dence from these data of a high correlation between retention on Pyralin plates and on leaves, as determined by the chemical-analysis procedure. The tenacity index also showed a significant correlation with the leaf retention, although, in 1941, the coefficient was not so high as that obtained by the direct-analysis method. The tenacity index was slightly better correlated with control than the direct analysis determination on plates although neither was significant. A low correlation was also found between retention on leaves and control.

The LD50 values for the treatments used in 1941 did show a significant correlation with the control data, suggesting that the toxicity of the material

rather than retention was the important factor influencing the protective value in the field. When the additional factor of retention was correlated with control and LD50 by means of multiple correlation, a slight increase in the coefficient was obtained. The highest multiple correlation coefficient obtained was between control, tenacity index, and LD50, and is high enough to be significant with the lower number of degrees of freedom (n – 3) required for 3 factors.

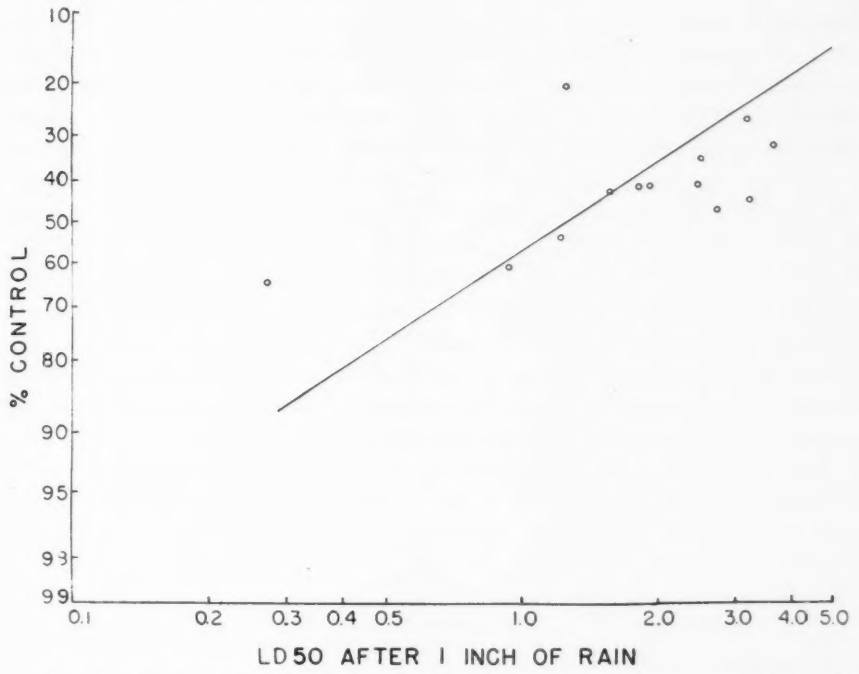

FIG. 2. LD50 values after rain plotted against control (percentage disease-free leaves) on logarithmic probability paper.

The relation between the LD50 after a rain test (LD50/tenacity index) and control expressed as disease-free leaves is shown in figure 2 on logarithmic probability paper. A distinct trend is noted excepting for two points. One of these points is Bordeaux mixture and the other is Cupro-K. The latter material has been found in laboratory tests to be much more soluble than the others and would probably explain its high toxicity. By ignoring this point (LD50 1.26) can be stated that this indicates that percentage control is normally distributed against the reciprocal of the logarithm of the LD50 after the rain test. Such a relationship emphasizes the need for LD50 values in the laboratory toxicity tests after being subjected to a laboratory rain.

DISCUSSION

The direct chemical analysis was used in the retention studies on Pyralin plates in the laboratory since it was assumed that this would make the deter-

minations more comparable with that obtained for the leaves in the field when this was the only practicable method to use. The high correlation between the retention on leaves and Pyralin plates indicates the validity of this assumption. However, the fact that there was so little correlation between retention of actual copper and control indicates that some other more important factor was operating to limit the protective value in the field. Toxicity of the material must have been this factor, since the LD50 values gave such a high correlation with control. Retention was involved, but was of much less importance than toxicity.

Although the 3 methods of evaluating retention correlated very well, certain exceptions were found for which no satisfactory explanation seems available. There are, of course, many factors that are different in the laboratory and field tests. Martin (20), for example, has pointed out that in field spraying many leaves are sprayed beyond the point of run-off, while this is not done in ordinary laboratory tests. Worthley and Frear (27) suggested that the application of more than one spray to the leaves probably caused some discrepancy between results on leaves and on Pyralin plates in a study of deposition and retention of lead arsenate. However, in some preliminary work with the copper fungicides used in this study, more variation as indicated by a much higher standard error for the original residue was found where only the post-harvest application of a copper material had been made than where 3 copper sprays had preceded this.

The fact that a cherry leaf falls as soon as it becomes heavily infected with leaf spot or injured with spray, tends to cause an increase in the percentage of leaves with a high residue on treatments giving inferior control and a lower residue on treatments that cause excessive injury. No injury was apparent in the 1940 and 1941 tests.

Difference in the chemical and physical nature between leaves and the surfaces used in the laboratory must be considered. The weathering process in the laboratory consists of washing with water alone; yet, in the field, mechanical abrasion (7), alternate wetting and drying, fluctuations in temperature, and sunlight, probably, also are involved. In fact, so many differences exist that it seems especially significant to have data that indicate high correlation between the two types of test.

The correlation between retention as determined by direct chemical analysis and, as determined by the spore germination technique, seems significant in view of the statement by Heuberger (12) that the latter method determines both the quantity and fungicidal value of the residue remaining after weathering. The correlation of the two methods with retention on leaves was good in 1940; yet the spore germination test did not correlate so well in 1941 with the actual quantitative measurements on either leaves or plates. Horsfall (13) also found that 2 spray deposits having the same quantity of copper may not inhibit spore germination to the same extent. Martin (21) has noted certain changes in the composition of Bordeaux mixture upon drying, which would be expected to change the fungicidal

value of the residue and also would interfere with a quantitative measurement of the residue by a method depending on spore-germination tests without necessarily affecting the actual amount of copper retained.

An indication of the error involved in field determinations of retention can be found in table 2 where treatments 6 and 20 are the same in 1941, yet the retention index on the leaves differs considerably. This emphasizes the importance of a statistical approach to the interpretation of field data.

A comparison of data on the commercial compounds used in this study with those reported by other workers is difficult, since there is no certainty that the composition is the same from year to year, even though the trade name remains unchanged. Heuberger (12) found that soya flour did not increase retention, which agrees with the results obtained with Tennessee "26" in 1941. Hartmann (10) reported Cupro-K and Compound (Copper) "A" were lower in retention than Bordow, and that these 3 were lower than Bordeaux mixture and Copper Hydro "40," which is in accord with the 1941 results reported here.

The host plant, according to Horsfall (13) does not alter the ranking of materials studied by laboratory methods. Martin (21), on the other hand, has pointed out the importance of the host plant in changing the protective value of a fungicide. However, high correlations found in this study indicate that the laboratory methods are fairly accurate in predicting the behavior of materials in the field, although it is evident that some few may not perform as expected.

SUMMARY

The retention of several protective copper fungicides was determined in the laboratory by the direct chemical analyses of Pyralin plates sprayed under standard conditions. This showed a very high correlation with direct analytical determination of retention of the same materials on cherry leaves in 2 different years. These 2 methods also correlated very well with a determination of retention by the spore-germination technique.

Bordeaux mixture and 2 formulae of tank-mix copper phosphate showed a higher retention than any of the commercial forms of copper materials. Summermulsion and Spredrite, Spralastic, and Orthex all increased the retention of Tennessee "26" on leaves, while soya flour and Nufilm reduced it. Cottonseed oil emulsion increased the retention of Cupro-K. Tennessee "34" was retained better than Tennessee "26" or Copper Hydro "40." The retention of Tennessee "26" was the same with dolomitic lime as with regular high calcium lime. Coposil, Copper "A" and Bordow were low in retention on foliage.

Very little correlation was found between control and retention of copper on cherry leaves in the field and retention on Pyralin plates in the laboratory or the tenacity index. There was a significant correlation between control and toxicity expressed as the LD50. A significant correlation between control, tenacity index, and LD50 also was found. The percentage control

expressed as disease-free leaves was found to be normally distributed against the logarithm of the LD50 values after the laboratory rain.

It is concluded that the laboratory methods of determining retention predicted fairly accurately the retention of the same materials in the field, but that toxicity was a much more important factor than retention in predicting the protective value of the fungicides.

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STUDIES OF THE SUSCEPTIBILITY OF FORAGE GRASSES TO CEREAL SMUT FUNGI. IV. CROSS-INOCULATION EXPERIMENTS WITH UROCYSTIS TRITICI, U. OCCULTA, AND U. AGROPYRI[1]

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INTRODUCTION

Plant pathologists and mycologists have long noted the morphological similarity within 3 species of *Urocystis*, causing flag smut of grasses, wheat (*Triticum aestivum* L.), and rye (*Secale cereale* L.), namely, *Urocystis agropyri* (Preuss) Schroet., *U. tritici* Koern., and *U. occulta* (Wallr.) Rab., respectively. Until recently, however, no similarity in host relationships had been demonstrated. McAlpine (12) tried repeatedly to cross-infect wheat and rye with their respective flag smuts, and, being unsuccessful, concluded that the two species (*U. tritici* and *U. occulta*) are distinct. Griffiths (5) inoculated seed of redtop (*Agrostis alba* L.), timothy (*Phleum pratense* L.), rye, and wheat with flag smut from wheat and rye. The inoculum from wheat infected only wheat, and that from rye infected only rye. Jarrett (10) was unsuccessful in repeated attempts to extend the host range of *U. tritici* to other cereals and to grasses, and concluded that the flag smut of wheat is strictly limited to wheat. Recently, however, there was reported (3) the results of the first partially successful attempts at cross inoculation within these 3 species.

All 3 species of *Urocystis* mentioned above occur in the United States. Flag smut of grasses and flag smut of rye (also improperly called stem smut)[3] have long been known in this country, but flag smut of wheat is of comparatively recent appearance, having been discovered in the middle west first by S. M. Zeller in 1918, and then by J. G. Dickson in 1919 according to Humphrey and Johnson (9), and to Tisdale *et al.* (16). In 1940 it was reported from central Washington by Heald and Holton (6).

The origin of flag smut of wheat in the United States has been a much-discussed matter. Brittlebank (1), according to Tisdale *et al.* (15), was of the opinion that the disease probably had been introduced with the 5,500,000 bushels of wheat exported to the United States from Australia in 1918. This

[1] Cooperative investigations of the Divisions of Forage Crops and Diseases, and Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture, with the Washington State Agricultural Experiment Station, Pullman, Washington, and the Soil Conservation Service, United States Department of Agriculture. Published with the approval of the Experiment Station Director as Scientific Paper No. 539.

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[3] The designation of "stem smut" should be reserved for those smuts that involve the stem only, such as is typified by *Ustilago hypodytes* (Sch.) Fries. Flag smut of rye sporulates chiefly in the leaves and leave sheaths, and, even though the culm and inflorescence may be involved, the typical striped or "flag" aspect is obvious.

opinion prevailed until it was pointed out by Tisdale *et al.* (16), in 1927, that actually the first collection of flag smut of wheat in this country was made in 1918. Tisdale *et al.* (16) explained that the disease had to be present in the middle west at sowing time in 1917 in order to infect the crop of 1918, but added that this did not preclude the possibility of flag smut having been introduced into this country along with earlier shipments of wheat or other products from Australia or some other country.

In 1938 the senior writer found squirrel-tail grass (*Sitanion jubatum* L.), seriously infested (50 per cent) with flag smut (*Urocystis agropyri*) in Klickitat County, Washington. No particular attention was paid to this incidence of flag smut in squirrel-tail grass until its discovery in wheat 2 years later in the same locality. This led to some conjecturing as to the possible relationship that might exist between the flag smut of squirrel-tail grass and that of wheat. Flag smut of grasses has been long prevalent throughout the country on a wide variety of grasses (2, 17), and since no adequate explanation has been provided as to the source of the recent outbreak of the wheat flag smut in Washington, as well as in the Middle West in 1918 and 1919, the question arose as to whether these outbreaks represented strains of the similar smut of grasses that were capable of attacking wheat. The morphologically identical flag smut of grasses occurs in the Northwest on many species of *Agropyron, Bromus, Elymus, Festuca, Hordeum, Melica, Poa,* and *Sitanion,* and if the host range over the United States as a whole be considered, other genera could be added. This being the case, it seems reasonable that cultivated wheat might also be included, especially if common susceptibility could be demonstrated. Evidence of this could be obtained only from cross-inoculation experiments, the results of which are presented in this paper, together with a discussion of their bearing on the whole problem of flag smut in the United States, especially with regard to host relationships.

METHODS AND MATERIALS

Inoculation of grasses was effected by processing the seed in an aqueous suspension of smut spores, under partial vacuum. Wheat and rye were inoculated by dusting the seed with spores. All tests with wheat and rye were run in the greenhouse, as were most of the grasses, although some were planted in the field. These latter were started in the greenhouse in wood veneer plant bands and later transplanted as seedlings to the field. Since spores of *Urocystis agropyri* germinate more slowly than spores of *U. tritici* and *U. occulta,* they were soaked in tap water in the refrigerator at 2–5° C. for several weeks before being used. No pre-soaking was necessary for *U. tritici* and *U. occulta.*

Inocula of *Urocystis agropyri* were obtained from collections made during the past several years. The infected plant parts were macerated in water and the spores thus freed were recovered by straining through cheesecloth. Two collections of *U. tritici* were used in these studies, one of which

came from Leavenworth, Kansas,[4] and the other from Goldendale, Washington. The inoculum of *U. occulta* was obtained from University Farm, St. Paul, Minnesota.[5]

RESULTS

Some greenhouse experiments of a very preliminary nature were carried out in 1940. One or more accessions of *Agropyron, Elymus, Hordeum,* and *Sitanion* spp. were inoculated with 3 collections of *Urocystis agropyri* and 1 of *U. tritici. Agropyron inerme* (Scribn. and Smith) Rydb., alone became infected with *U. tritici,* while *A. trachycaulum* (Link) Malte, *A. spicatum* (Pursh) Scribn. and Smith, *A. inerme, Elymus glaucus* Buckl., *E. canadensis* L., *Sitanion jubatum,* and *S. hystrix* (Nutt.) J. G. Smith were infected with *U. agropyri.*

GREENHOUSE-FIELD PLANTINGS

Seed of many species of *Agropyron, Bromus, Elymus, Festuca, Hordeum, Lolium,* and *Sitanion* was inoculated with *Urocystis agropyri* (from *Hordeum nodosum,* Corvallis, Oregon), two collections of *U. tritici* and one of *U. occulta.* The inoculated seed was planted in the greenhouse in late winter (1941) and the seedlings were transplanted to the field early the following spring.

TABLE 1.—*Results of inoculations of grasses with flag smut of wheat, flag smut of rye, and flag smut of grasses (Urocystis tritici, U. occulta, and U. agropyri respectively)*[a]

Species	Acc. No.[b]	Source and identity of inoculum			
		Urocystis agropyri	*Urocystis tritici*		*Urocystis occulta*
		Oregon	Washington	Kansas	Minnesota
Agropyron caninum	F 138	--[c]	−	+	−
Agropyron caninum	F 139	+	+	+	+
Agropyron inerme	W 2522	−	−	−−	+
Agropyron repens	F 140	−−	+	+	−
Agropyron semicostatum	W 2903	−−	+	+	−
Agropyron spicatum	W 736	−−	+	−	−
Agropyron spicatum	W 739	−−	−	+	−
Elymus canadensis	W 786	−−	−	−	+
Elymus canadensis	W 788	+	−	−−	+
Elymus canadensis	W 2389	+	−	−−	+
Elymus canadensis	W 3549	+	−	−−	−
Elymus canadensis	W 4669	+	−	−−	−
Elymus glaucus	W 1753	−−	+	−	−
Elymus glaucus	W 2662	−	+	−−	−
Elymus triticoides	W 2741	−−	+	−	−

[a] Many species of *Bromus, Festuca, Hordeum, Lolium,* and *Sitanion* were included also but did not become infected. For the sake of brevity these are not listed.

[b] "F" numbers are those of the senior writer. "W" numbers are those of the Soil Conservation Service, United States Department of Agriculture, Pullman Nursery Unit.

[c] −− = not inoculated.
+ = typical flag-smut symptoms developed.
− = no signs of infection.

[4] Supplied by A. G. Johnson, Bureau of Plant Industry.
[5] Supplied by J. J. Christensen.

The results of this experiment are presented in table 1, where only those species are listed that proved susceptible to one or more of the flag smut collections. The perennial nature of this infection was shown by the fact that plants observed to be infected in 1941 showed flag smut again in 1942. The percentages of infection were quite variable, ranging from a trace to nearly 50 per cent (*U. occulta* on *E. canadensis*).

EXPERIMENTS IN THE GREENHOUSE

Where there was sufficient spore material, inoculum was prepared from the above infected grasses (Table 1) infected with flag smuts of wheat, rye,

TABLE 2.—*Results of inoculations of wheat, rye, and grasses with flag smut (Urocystis agropyri, U. tritici and U. occulta) taken from grasses artificially infected with flag smut*

Smut	Original source	Immediate source	Grass or cereal inoculated	Per cent[b] smut
U. agropyri	*Hordeum nodosum*, Oregon	*Elymus canadensis* W 4669	*Elymus canadensis* W 4669	3.4
U. agropyri	*Hordeum nodosum*, Oregon	*Elymus canadensis* W 2389	*Elymus canadensis* W 2389	8.5
U. agropyri	*Hordeum nodosum*, Oregon	*Elymus canadensis* W 3549	Wheat[a]	0.0
U. agropyri	*Hordeum nodosum*, Oregon	*Elymus canadensis* W 3549	Rye (Prolific)	0.0
U. tritici	Wheat, Washington	*E. glaucus* W 1753	*E. glaucus* W 1753	34.7
U. tritici	Wheat, Washington	*E. glaucus* W 1753	Wheat	33.7
U. tritici	Wheat, Washington	*A. caninum* F 139	*A. caninum* F 139	0.0
U. tritici	Wheat, Washington	*A. caninum* F 139	Wheat	20.4
U. tritici	Wheat, Washington	*E. triticoides* W 2741	*E. triticoides* W 2741	0.0
U. tritici	Wheat, Washington	*E. triticoides* W 2741	Wheat	39.4
U. tritici	Wheat, Washington	*A. repens* F 140	*A. repens* F 140	1.4
U. tritici	Wheat, Washington	*A. repens* F 140	Wheat	11.4
U. tritici	Wheat, Washington	*A. semicostatum* W 2903	*A. semicostatum* W 2903	5.3
U. tritici	Wheat, Washington	*A. semicostatum* W 2903	Wheat	14.2
U. tritici	Wheat, Kansas	*A. caninum* F 138	*A. caninum* F 138	13.7
U. tritici	Wheat, Kansas	*A. caninum* F 138	Wheat	20.8
U. tritici	Wheat, Kansas	*A. spicatum* W 739	*A. spicatum* W 739	5.0
U. tritici	Wheat, Kansas	*A. spicatum* W 739	Wheat	26.1
U. tritici	Wheat, Kansas	*A. repens* F 140	*A. repens* F 140	3.7
U. tritici	Wheat, Kansas	*A. repens* F 140	Wheat	12.0
U. occulta	Rye, Minnesota	*E. canadensis* W 788	*E. canadensis* W 788	4.3
U. occulta	Rye, Minnesota	*E. canadensis* W 788	Rye (Dakold)	32.8
U. occulta	Rye, Minnesota	*E. canadensis* W 2389	*E. canadensis* W 2389	14.2
U. occulta	Rye, Minnesota	*E. canadensis* W 2389	Rye (Dakold)	35.4
U. occulta	Rye, Minnesota	*A. caninum* F 139	*A. caninum* F 139	0.0
U. occulta	Rye, Minnesota	*A. caninum* F 139	Rye (Prolific)	8.3

[a] All wheat listed in this column is Kanred × Hard Federation (C.I. 10092).
[b] Infection percentages based on a range of from 7–215 plants per row, with an average of at least 90.

and grasses, and used to reinoculate their natural hosts. All cereal and grass seed thus inoculated was sown in rows in the greenhouse October 13, 1941. The first symptoms on both grasses and cereals were observed November 29; and on December 26 notes were taken. The results of these inoculations are presented in table 2.

The results (Table 2) fully substantiate those of the field sowings in which several species of grasses were infected with *Urocystis tritici* and *U. occulta*. In every case, excepting check rows, infection was obtained on rye inoculated with *U. occulta* from the artificially infected grasses, and on wheat with *U. tritici* from artificially infected grasses. The fact that the infection percentages on the cereals were generally so much higher than on the grasses leaves little room for any doubt that the apparent infection of the grasses with the cereal flag smut is valid.

Considering the foregoing results of the preliminary inoculations of grasses with flag smut, it seemed desirable to follow up with a more exhaustive test, using several recent collections of *Urocystis agropyri* in addition to *U. tritici* and *U. occulta*. Following the same technique already outlined, seed of the following grass species was inoculated with 4 collections of *U. agropyri*, 2 of *U. tritici*, and 1 of *U. occulta*: *Agropyron cristatum* (L.) Gaert., *A. dasystachyum* (Hook.) Scribn., *A. desertorum* (Fisch.) Schult., *A. elmeri* Scribn., *A. elongatum* (Host.) Beauv., *A. inerme*, *A. repens*, *A. semicostatum*, *A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb., *A. spicatum*, *A. subsecundum* (Link) Hitchc., *A. trachycaulum*, *A. trichophorum* (Link) Richt.; *Elymus canadensis*, *E. glaucus*, *E. sibiricus* L., *E. triticoides*; *Hordeum brevisubulatum* (Trin.) Link, *H. bulbosum* L., *H. jubatum* L., *H. jubatum* var. *caespitosum* (Scribn.) Hitchc., *Poa ampla* Merr., *P. nevadensis* Vasey, *P. pratensis* L., *P. secunda* Presl.; *Sitanion jubatum*. In addition to the above grasses the following cereals were included: Hard Federation wheat (C.I. 4733), Kanred × Hard Federation wheat (C.I. 10092), Prolific rye, Dakold rye, and Rosen rye × *Secale montanum* Guss.[6] The inoculated seed was planted in rows in greenhouse benches, January 1, 1942. On February 3 the first symptoms were noticed in several of the grasses. It was observed at that time, and has been noticed repeatedly since, that flag smut apparently does not appear earlier than on the third seedling leaf. Data were taken beginning March 14, 1942.

The positive results of the above inoculations of grasses and cereals with seven flag smut collections are given in table 3. The grass-host range of both *Urocystis tritici* and *U. occulta* is extended by the above experiment. New hosts for the wheat flag smut are *Agropyron dasystachyum*, *A. desertorum*, *A. trachycaulum*, *Elymus canadensis*, and *Hordeum jubatum* var. *caespitosum*. *Elymus triticoides* is a new host for *U. occulta*. These results present the first published experimental evidence of the existence of physiologic specialization in the flag smut of grasses, *Urocystis agropyri*. Collections C–I, C–H, and C–F, from *Agropyron trachycaulum*, *Hordeum jubatum*, and

[6] Cross by D. C. Smith, formerly of the Bureau of Plant Industry.

Flag smut collections[b]

tions of flag smut (*Urocystis agropyri*, *U. occulta* and *U. tritici*) in the Greenhouse 1942[a]

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Hosts	Flag smut collections[b]													
	Urocystis agropyri								*Urocystis occulta*		*Urocystis tritici*			
	C–I		C–H		C–G		C–F		C–E		C–D		$C–D_1$	
	Plants	Smut	Plants	Smut	Plants	Smut	Plants	Smut	Plants	Smut	Plants	Smut	Plants	Smut
Grasses:	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
Agropyron dasystachyum W 4702[c]	45	17.7	49	2.0	59	0.0	34	0.0	21	0.0	43	0.0	63	11.1
Agropyron dasystachyum W 7801	81	9.8	69	2.8	115	0.0	24	0.0	48	0.0	29	0.0	102	0.9
Agropyron desertorum W 11	186	0.0	65	0.0	97	0.0	26	0.0	35	0.0	16	0.0	74	1.3
Agropyron elongatum W 2326	101	0.9	90	0.0	130	0.0	62	0.0	98	0.0	92	0.0	94	0.0
Agropyron inerme W 2522	107	4.6	81	0.0	135	0.0	26	0.0	36	0.0	31	0.0	47	0.0
Agropyron inerme W 2617	95	6.2	72	0.0	204	0.0	28	0.0	19	0.0	29	0.0	44	2.2
Agropyron repens F 140	67	0.0	57	0.0	72	0.0	56	0.0	39	0.0	31	0.0	99	3.0
Agropyron semicostatum W 2903	52	3.8	50	0.0	74	0.0	27	3.7	27	0.0	51	0.0	111	0.0
Agropyron spicatum W 736	18	22.2	28	0.0	41	0.0	12	0.0	9	0.0	17	0.0	34	0.0
Agropyron spicatum W 739	30	3.3	32	0.0	18	0.0	3	0.0	4	0.0	8	0.0	26	0.0
Agropyron subsecundum F 13	71	5.6	83	0.0	87	0.0	50	0.0	58	0.0	66	0.0	123	0.0
Agropyron trachycaulum F 67	28	0.0	22	0.0	83	0.0	70	0.0	46	0.0	75	0.0	128	0.7
Elymus canadensis W 2389	75	13.3	66	0.0	69	0.0	67	13.4	41	17.0	70	0.0	66	9.0
Elymus canadensis W 788	33	51.5	72	0.0	91	0.0	86	17.4	60	13.3	86	0.0	74	2.7
Elymus glaucus W 1753	36	11.1	45	0.0	58	0.0	33	0.0	15	0.0	40	10.0	48	25.0
Elymus glaucus W 2662	35	0.0	80	0.0	60	0.0	52	0.0	47	0.0	70	0.0	78	5.1
Elymus sibiricus W 225	54	1.8	25	0.0	55	0.0	53	0.0	39	0.0	46	0.0	78	0.0
Elymus triticoides W 2741	60	0.0	61	0.0	49	0.0	18	0.0	28	3.5	55	0.0	65	0.0
Elymus triticoides W 3250	40	0.0	59	0.0	67	0.0	20	5.0	49	0.0	56	0.0	85	0.0
Hordeum jubatum var. *caespitosum* F 334	106	0.9	72	4.1	75	0.0	66	0.0	49	0.0	87	1.1	52	3.8
Sitanion jubatum W 3360	33	18.1	62	0.0	45	0.0	25	0.0	17	0.0	16	0.0	11	0.0
Cereals:														
Hard Federation wheat	40	0.0	42	0.0	34	0.0	32	0.0	32	0.0	41	17.0	81	12.3
Kanred × Hard Federation	47	0.0	50	0.0	43	0.0	43	0.0	43	0.0	46	21.7	84	15.4
Prolific rye	18	0.0	19	0.0	24	0.0	15	0.0	22	9.0	25	0.0	25	0.0
Dakold rye	41	0.0	37	0.0	44	0.0	32	0.0	29	24.1	39	0.0	70	0.0
Rosen rye × *Secale montanum*	24	0.0	14	0.0	12	0.0	12	0.0	14	7.1	9	0.0	6	0.0

[a] For the sake of brevity species not infected by any of the seven collections of flag smut are omitted from this table.

[b] These collections of flag smut originated as follows:

C–I *Urocystis agropyri* from *Agropyron trachycaulum* Yellowstone Park, Wyoming.
C–H *Urocystis agropyri* from *Hodreum jubatum*, Baker, Oregon.
C–G *Urocystis agropyri* from *Poa ampla*, Yellowstone Park, Wyoming.
C–F *Urocystis agropyri* from *Agropyron repens*, University Farm, St. Paul, Minn.
C–E *Urocystis occulta* from cultivated rye, St. Paul, Minnesota.
C–D *Urocystis tritici* from cultivated wheat, Goldendale, Washington.
$C–D_1$ *Urocystis tritici* from cultivated wheat, Leavenworth, Kansas.

[c] "W" numbers are the accession numbers of the Pullman Nursery Unit of the Soil Conservation Service, U. S. Department of Agriculture.

Agropyron repens, respectively have quite different host ranges and probably represent 3 different races. No satisfactory definite explanation can be offered for the behavior of collection C–G, from *Poa ampla*. This collection failed to infect any of the 28 species of grasses (including *Poa ampla* and other *Poa* spp.) inoculated, as well as the cereals. Either all of these were immune from collection C–G, or, what seems more likely, the inoculum did not contain germinable spores.[7]

The 2 collections of *Urocystis tritici*, C–D and C–D_1, have previously been shown (8) to represent 2 physiologic races, by their reactions on wheat. This fact is confirmed by their reactions on grasses (Table 3). On the wheat differentials the Washington race is more widely virulent than the Kansas race; whereas, on grasses, the Kansas race is considerably more virulent than the Washington race. A higher percentage of infection was, in fact obtained on *Elymus glaucus* with the Kansas race than on the 2 wheat varieties.

The flag smut of rye did not appear highly virulent on grasses; in fact, it seems that a fair test of the susceptibility of the 28 grass species to all 7 flag smut collections may not have been obtained; as, in general, the percentages of infection are low. Even with the inoculations of *U. tritici* on known susceptible wheats, and of *U. occulta* on susceptible varieties of rye, the percentages of infection were comparatively low, none exceeding 24.1 per cent. This would indicate that some unknown factors may have operated against infection in the entire experiment.

Cross-inoculation Experiments with Urocystis agropyri, U. tritici, and U. occulta on Cereals and Cereal × Grass Hybrids

In a preliminary test (1940–41) on the susceptibility of wheat and rye to the flag smut of grasses (*Urocystis agropyri*) 32 varieties of wheat and 2 of rye were inoculated with a composite of collections from *Elymus canadensis* and *Agropyron spicatum*. The results were negative. In a second experiment of the same nature (1941–42) 7 varieties of wheat, known to be susceptible to *U. tritici* and 2 varieties of rye susceptible to *U. occulta* were inoculated with *U. occulta* and a composite of collections of *U. agropyri* from various grasses.[8] Good infection resulted on the rye inoculated with *U. occulta;* but, aside from this, the results were negative. Then the experiment was repeated, using a mixture of collections of *U. agropyri* from *Elymus canadensis* and *Agropyron spicatum*. This time, 2 of 30 plants of Kanred × Hard Federation wheat resulting from seed inoculated with *U. agropyri* showed typical flag-smut symptoms. These infected plants were dried and the spores obtained from them were used to reinoculate Kanred × Hard Federation and also *Elymus glaucus*, resulting in 17 and 21 per cent infection, respectively.

[7] The inoculum was not checked for percentage of spore germination. As yet no germinating spores of *Urocystis agropyri* have been observed, although preliminary attempts have been made to induce germination by the technique employed with *U. tritici* and *U. occulta* (11, 13, 14).

[8] Collections C–I, C–H, C–G, and C–F. See footnote b, table 3.

Inasmuch as various cereal × grass hybrids were available through the courtesy of cooperating agencies, a number of these hybrids and their parents were inoculated with *Urocystis tritici* and *U. occulta*. The results are shown in table 4.

TABLE 4.—*The results of inoculating certain cereal × grass hybrids and their parents with Urocystis tritici and U. occulta in the greenhouse*

Host	*U. tritici*	*U. occulta*
Secale montanum	– –[a]	– –
Elymus condensatus	–	–
Dakold rye × *S. montanum* 3–46. F_1 seed	+	+
Rosen rye × *S. montanum* 22–12. F_1 seed	–	+
Prolific rye × *S. montanum* 5–14. F_1 seed	+	+
Michel's rye	–	+
Dakold rye	–	–
Mosida wheat	+	–
Prolific rye	–	+
Rosen rye	–	+

[a] – – = no stand obtained.
– = no infection.
+ = typical flag smut symptoms. (Counts were not made and percentages of infection were not calculated.)

As seen in table 4, 2 of the hybrids (Dakold rye × *Secale montanum* F_1 and Prolific rye × *S. montanum* F_1 were infected with both *Urocystis tritici* and *U. occulta*. It was not surprising to obtain infection on these hybrids with the rye flag smut but the infection with the wheat flag smut was worthy of notice. Repeated attempts to infect the rye parents, Dakold and Prolific rye, with flag smut of wheat have been unsuccessful. This would indicate susceptibility in the other parent, *Secale montanum*. Unfortunately no stand was obtained with this grass, and this possibility was not tested. The susceptibility of *S. montanum* to the flag smut of rye, however, was demonstrated as early as 1907 by Hecke (7), according to Noble (13).

The results of the experiment on which table 4 is based, not the least of which was the infection of Michel's rye with *Urocystis occulta*, prompted a repetition on a slightly larger scale. *Urocystis agropyri* (a composite of collections from *Agropyron*, *Hordeum*, and *Poa* spp.) was added to the smuts, and other hybrids and parents added to the grasses. The results of this second experiment are presented in table 5.

The results shown in table 5 at least partly substantiate the results shown in table 4. Only one of the rye × *Secale montanum* hybrids was infected with *Urocystis tritici*, and surprisingly enough, it was not infected with *U. occulta*. However, this was not the same hybrid as was used before (Table 4), although the parents are the same. The other hybrid that became infected with both *U. tritici* and *U. occulta* in the previous test was this time not infected by either species. Again Michel's rye showed some infection by *Urocystis occulta*. Three other rye × *Secale montanum* hybrids, not included in the previous test, were infected with the rye flag smut. For the second time, *Elymus condensatus* appeared to be immune from both the

TABLE 5.—*Results of inoculations of cereal × grass hybrids and their parents with Urocystis tritici, U. occulta, and U. agropyri in the greenhouse*

Host	*Urocystis tritici*		*Urocystis occulta*		*Urocystis agropyri*	
	Total plants	Smut	Total plants	Smut	Total plants	Smut
	No.	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
Secale montanum	--[a]	--	--	--	--	--
Elymus condensatus	20	0.0	15	0.0	30	0.0
Dakold rye × *S. montanum* F_2	47	6.8	20	0.0	35	0.0
Rosen rye × *S. montanum* F_2	45	0.0	15	0.0	40	0.0
Prolific rye × *S. montanum* F_1	18	0.0	6	0.0	18	0.0
Michel's rye	25	0.0	18	5.5	35	0.0
Dakold rye	87	0.0	12	0.0	20	0.0
Mosida wheat	21	0.0	26	0.0	30	0.0
Prolific rye	20	0.0	12	16.6	25	0.0
Rosen rye	10	0.0	8	50.0	20	0.0
Spring rye × *S. montanum*	40	0.0	19	15.7	45	0.0
S. cereale × *S. montanum*–10	12	0.0	4	25.0	17	0.0
S. cereale × *S. montanum*–4	1	0.0	1	100.0	10	0.0
Wheat × *Agropyron elongatum*	7	0.0	13	0.0	20	0.0
Agropyron elongatum	30	0.0	50	0.0	50	0.0

[a] -- No stands of *Secale montanum* were obtained.

wheat and the rye flag smuts. Mosida wheat did not this time become infected with *U. tritici*, as it had before.[9]

Urocystis agropyri produced no visible symptoms on any of the hybrids and parents tested.

DISCUSSION

The foregoing results indicate a genetic relationship between the flag smut of grasses, *Urocystis agropyri*, and the flag smut of wheat, *U. tritici*. Possibly the flag smut of rye, *U. occulta*, is also involved, but the latter is morphologically distinct, whereas *U. agropyri* and *U. tritici* are indistinguishable on that basis (4). The established susceptibility of several common grasses to *U. tritici* indicates that native and introduced grasses could very easily support the wheat flag smut in nature, a fact which could eventually become a factor in the control of the disease on wheat. The present results indicate very little susceptibility in wheat to the flag smut of grasses. Still, the slight infection of Kanred × Hard Federation by *U. agropyri* gives a hint that from the nation-wide grass flag smut there could have arisen strains that are capable of attacking wheat in addition to grasses. Thus, the outbreaks of wheat flag smut in the middle west and in the far west may have had an indigenous origin, rather than having been introduced

[9] The parentage of Michel's rye has been reported as *Elymus condensatus* × Mosida wheat, but it has been contended that the parentage is actually rye × *Secale montanum*. If it were a hybrid between wheat and *E. condensatus*, perhaps some susceptibility to wheat flag smut should be manifest, especially since the wheat parent became infected in the first test. Instead, Michel's rye has shown at least some susceptibility to the rye flag smut in both tests and no susceptibility to wheat flag smut. This might be interpreted as additional evidence that Michel's rye is of rye parentage, rather than of wheat. It has behaved, in these experiments, much like the rye × *Secale montanum* hybrids used.

from Australia or elsewhere. The hypothesis finds some support in the high degree of susceptibility of certain common native grasses (*e.g., Elymus canadensis* and *E. glaucus*) to the flag smut of wheat in the United States. Repeated attempts in Australia to infect grasses with the wheat flag smut having been unsuccessful (10), it is possible that a different race is prevalent in that country.

This hypothesis that flag smut of wheat (in the United States) arose as strains of *Urocystis agropyri* capable of attacking wheat may not seem very strongly supported by the results presented in this paper. However, too few collections of the grass flag smut have been studied for the idea to be abandoned without further test.

If the slight infection of Kanred × Hard Federation wheat by *Urocystis agropyri,* described in the results above, is experimental evidence that flag smut of wheat, in the United States at least, represents strains of *U. agropyri* capable of infecting wheat, then the smut resulting from inoculation of Kanred × Hard Federation wheat with the bulk collection of *U. agropyri* might represent a "strain" of *U. agropyri* that had been "screened out" on this wheat variety and that should be capable of continued propagation on the same variety. This is exactly what happened. Where only 6 per cent infection had been obtained when Kanred × Hard Federation was inoculated with the composite collection of *U. agropyri,* 17 per cent infection resulted when this wheat variety was re-inoculated with inoculum made up from the original 6 per cent infection.

It might be argued that there is no greater justification for an hypothesis that flag smut of wheat in the United States derived from the flag smut of grasses than that the converse could have happened. However, the flag smut of grasses has been known in this country for 3 or 4 decades longer than flag smut of wheat and is widely distributed from coast to coast, whereas the wheat smut is localized in a few areas in the middle west and in one small area in the far west.

The present results indicate that within the flag smut of grasses, *Urocystis agropyri,* there probably exist many physiologic races. Three of four collections included in these studies proved to represent different races. These results would seem to strengthen the suggestion recently made (3, 4) that the flag smut of wheat, *U. tritici,* be considered a specialized variety of *U. agropyri.* There does not seem to be any more reason to recognize the flag smut of wheat as a distinct species than any of the other morphologically identical races on Gramineae.

From the results of the cross-inoculation experiments to date it appears that the flag smut of rye has much fewer grass hosts than has that of wheat. Thus far only 2 species of *Agropyron* and 2 of *Elymus* have shown any susceptibility to flag smut of rye; whereas 8 species of *Agropyron,* 3 of *Elymus,* and 1 of *Hordeum* have shown more or less susceptibility to the flag smut of wheat. McAlpine (12) reported *U. occulta* on *Poa caespitosa* Forst. and *Lolium perenne* L. in Australia but these records are subject to suspicion as

referring to *U. agropyri* rather than to *U. occulta*. McAlpine's (12) illustrations of the spore balls of *U. occulta* on *Poa caespitosa* show the spores completely invested by the sterile cells, which would indicate that the smut was *U. agropyri*. McAlpine (12) also stated that *U. occulta* occurs on oats and barley, but did not indicate any authority for such records.

SUMMARY

The results are reported of cross-inoculation experiments with the flag smut of grasses (*Urocystis agropyri*), the flag smut of wheat (*U. tritici*), and the flag smut of rye (*U. occulta*), and the susceptibility of forage grasses to these smuts.

Twelve species of grasses have been found more or less susceptible to the flag smut of wheat. These are *Agropyron caninum, A. dasystachyum, A. desertorum, A. inerme, A. repens, A. semicostatum, A. spicatum, A. trachycaulum; Elymus canadensis, E. glaucus, E. triticoides;* and *Hordeum jubatum* var. *caespitosum*.

Four species of grasses have exhibited more or less susceptibility to flag smut of rye. These species are *Agropyron caninum, A. inerme; Elymus canadensis*, and *E. triticoides*.

There is evidence of considerable host specialization in the flag smut of grasses and many physiologic races probably occur in the United States. Three of 4 collections studied proved to represent different races.

One variety of wheat (Kanred × Hard Federation C.I. 10092) exhibited slight susceptibility to *Urocystis agropyri* in 1 out of 3 trials, and the smut thus produced was easily propagated on the same wheat variety. Wheat and rye seem to be immune from *U. occulta* and *U. tritici*, respectively. Rye seems immune from *U. agropyri*.

It is considered that the present results indicate a genetic relationship between *Urocystis agropyri* and *U. tritici*. *U. occulta* may also be involved. Considering the number of grasses that already have exhibited at least slight susceptibility to flag smut of wheat, it is suggested that flag smut of wheat may have arisen as strains of *U. agropyri* capable of attacking wheat, and that, therefore, the outbreaks of wheat flag smut in the United States may have an indigenous origin from the widespread flag smut of grasses, rather than having been introduced from abroad.

The data presented herein concerning the host relationships of the three flag smuts substantiate the morphological identity already demonstrated and strengthen the recommendation that the flag smut of grasses and the flag smut of wheat be consolidated under the binomial having priority, which is *Urocystis agropyri* (4).

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ESTIMATION OF THE LEAF AREA OF POTATO PLANTS FOR PATHOLOGICAL STUDIES

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INTRODUCTION

Measurement of the leaf area of experimental plants is normally a laborious process, often involving the destruction of the plants on which measurements are taken. A method recently has been devised[2] that greatly facilitates such measurements, that can be applied without injury to experimental plants, and that is as accurate as some of the more laborious methods. A series of standards is obtained by making tracings of leaves graded in size from the smallest to the largest, the tracings are numbered serially, and each leaf on the plants to be measured is matched with the standard nearest in size. The area of the leaf is recorded by applying to it the number of the corresponding standard.

The principle of this method has been modified and extended to the measurement of whole plants. In an experimental plot, the areas of all leaves on a few plants differing in size are compared with a set of standard leaves, the total area for each plant is referred to a scale covering the whole range of plant size, and the plants are given numbers according to their position on the scale. They are then used as standards for estimating the size of all plants in the plot. In practice, once the reference plants are given numbers according to the scale, the erection of a full series of standards becomes more an act of judgment and memory than of comparison.

This method, if properly applied, is very quick and surprisingly accurate. A practised operater, after establishing the scale of sizes by measurement and comparison, can walk through a plot calling the numbers that indicate plant size almost as quickly as they can be written down. By far the most laborious part of the method is the assembly, conversion, and analysis of the data.

The adaptability of the method has been proved by applying it to potato plots containing a number of varieties varying widely in growth habit. After a little practice, the mental adjustment necessary when passing from one variety to another becomes almost entirely mechanical.

The method is subject to two kinds of errors, errors of judgment, and errors arising from the grouping of the leaf areas into classes. The extent of the former may be tested in two ways: by measuring the areas of plants with a set of leaf standards after the plants have been rated, and by making and comparing duplicate ratings. The errors of grouping are equivalent to those involved in the grouping of data for statistical analysis, and will be

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[2] Thirumalachary, N. C. A rapid method of measurement of leaf areas of plants. Ind. Jour. Agr. Sci. **10**: 835–841. 1940.

small, provided sufficient classes are established, and the data are well distributed amongst the classes.

The success or failure of the method depends on how great are the errors of judgment in comparison with the accuracy demanded by the nature of the experiment.

DESCRIPTION OF THE EXPERIMENTAL PLANTS

Leaf areas for whole plots of potato plants were needed for two types of experiment, i.e., on transmission of leaf roll by aphids and on yield. The method of obtaining the leaf area was developed by trials mainly on a block of plants set out for investigations on the aphis transmission of leaf roll. The majority of examples and results given in the following description are drawn from the data for this block.

The block consisted of 864 plants in 16 rows of 54, and was surrounded by check rows to eliminate edge effects. The experiment was designed to include four varieties, Early Carman,[3] Western Australian Delaware (early varieties), Up-to-Date, and Tasmanian Brownell. The statistical unit subplot was intended to consist of 5 plants of the same variety and an Up-to-Date plant known to be infected with leaf roll, but when the plants were growing, it was found that the healthy Up-to-Date stock contained about 40 per cent. of another variety, probably Great Scot.

The first attempt to obtain a figure for leaf area was semi-quantitative, and was made on October 9th. Five other ratings were made, the last on December 11th. At this time, both early and late varieties had completed the period of most rapid vegetative growth and were developing tubers.

TABLE 1.—*Ratings and corresponding leaf areas for the scale used in estimating areas of potato leaves*

Rating	Log leaf area	Leaf area
	sq. cm.	*sq. cm.*
< 1	1.0	10
1	1.32	21
2	1.425	27
3	1.53	34
4	1.635	43
5	1.74	55
6	1.845	70
7	1.95	89
8	2.055	114
9	2.16	145
10	2.265	184
11	2.37	237
12	2.475	299

DEVELOPMENT OF A STANDARD FOR LEAVES

In obtaining a standard series of outlines, a number of leaves varying in size, shape, and variety, were picked from plants of the same varieties as

[3] For a discussion of the origin of these varieties, see C.S.I.R. (Australia) Pamphlet 106.

were in the experimental block, and contact prints were made of them on photographic paper. Reasonably clear prints were obtained, without injuring or distorting the leaves, by placing them with the midrib lying in a channel between two sheets of glass placed slightly apart on the screen of a printer. Pressure could then be exerted to bring the paper and the leaf into contact without crushing the leaf.

When the prints of a number of leaves were made, they were laid out on a bench. Three of the smallest were chosen because they appeared to belong to an evenly ascending series of sizes and rated 1, 2 and 3. Amongst the prints available there did not appear to be a fourth that was bigger than 3 by the same amount as 3 was bigger than 2 or 2 than 1. Similarly, there was none that seemed of a size to be rated 5, but there was one that could be rated 6; and from there the series was continued up to 8 by the choice of two successively larger leaves. The areas of the chosen prints were measured with a planimeter and compared with the ratings. The results are shown in figure 1, a. Other attempts at rating gave similar results.

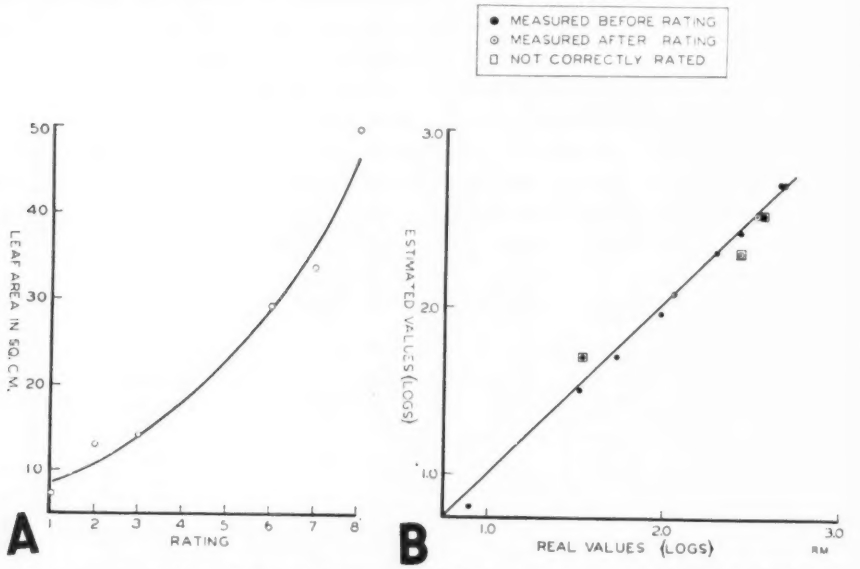

FIG. 1. A. Rating of six potato leaves and their areas as measured with a planimeter. The curve passing through the points was obtained from a straight line fitted to the values for log leaf area by the method of least squares. B. Comparison of leaf areas of whole plants which were both measured by means of the leaf standards (real values) and rated according to the standard scale given in table 3 (estimated values).

It was obvious that the points did not fit well into an arithmetic series, but when logs of the areas for a series of ratings were plotted, the values fell about a straight line. Apparently the mind judges leaf area on a basis of proportionality, *i.e.*, by the addition or substraction of equal relative increments rather than of absolute increments.

This basis appeared to offer a convenient means of compressing a wide range of sizes into a workable scale. Prints of leaves were measured until

samples were obtained that fitted into the 12-point scale shown below (Table 1). This scale was not too coarse for the kind of work contemplated, and it was quick in use and convenient to handle. The prints chosen were pasted on cloth sheets, supported on a stiff board, and bound at the top.

No leaves were found that were too large to be rated 12, but there was a number smaller than the leaf rated 1. An area of 10 sq. cm. was ascribed to all these leaves, and to the whorls of young leaves at the growing tip.

To remove the chance of bias in matching the leaves because of differences in shape, the more dissected types were chosen for odd-number ratings, and the more rounded and less dissected types for the even numbers. A leaf of any shape could then be placed for comparison between two somewhat similar outlines. The alternation of different shapes on the scale also helped to obviate the need for constructing different scales for different varieties.

DEVELOPMENT OF A STANDARD FOR PLANTS

The first two ratings of this block of plants were made on October 9 and 16. Plants were noted as "not yet up," "aboveground but the first young leaves not fully expanded," and "the first young leaves expanded." This classification was given quantitative expression by assuming that the second and third classes were equivalent to the first two on a scale developed for the third rating made on October 24 (Table 2). The areas so obtained were not grossly incorrect, and they gave a reasonable picture of the relative leaf areas of the different varieties at each date.

On October 24 the plants were rated on a 10-point scale by choosing the largest and some of the smallest plants, rating them 10 and 1, and ascribing what seemed appropriate values to plants of intermediate size. After the rating was made, plants of various sizes and varieties were chosen at random, and the leaf areas were found by measurement with the leaf standards. The results were somewhat irregular, but they did not suggest that estimation of the leaf area of whole plants was made on a simple basis of proportionality.

A week later, the largest plant had nearly doubled its leaf area so an attempt was made to double the intervals on the scale for rating. The result was fairly successful; the largest plant was rated 9 and the intermediate ratings were about double the previous ratings. Again the estimates of leaf area could not be fitted on a simple basis of proportionality, but neither did the increments appear always to be equal on an arithmetical scale although they were near enough to equality to make the use of an arithmetical scale permissible. The values shown in table 2 were used in converting the ratings to areas. The values for 24.10.42 and 31.10.42 were obtained from straight lines fitted to experimental values on a graph of the measured leaf area plotted against the rating previously given to the measured plants. Immediately after the ratings in the plot were completed, a number of plants of various sizes were chosen at random, and their leaf areas measured by means of the leaf standards. For each plant the area so obtained was

plotted against the rating. Straight lines were fitted to the points on the graph.

The lines that gave the values in table 2 varied very slightly, but far from significantly, from those obtained by the method of least squares: the increments gave the nearest rounded figures to the figures calculated.

TABLE 2.—*Scales used in converting ratings of plant size into leaf areas. Leaf Roll Transmission Exp. Block 1, 1941–42*

Date:	24.10.41		31.10.41			12.11.41	
Rating	[a]Area in sq. dm.		Rating	[a]Area in sq. dm.		Rating	Area in sq. dm.
	3 varieties	Brownell		3 varieties	Brownell		
1	.5	0.4	1	2.25	1.25	1	6.2
2	3.0	2.0	2	7.25	5.0	2	16.5
3	5.5	3.6	3	12.25	8.75	3	18.7
4	8.0	5.2	4	17.25	12.5	4	21.4
5	10.5	6.8	5	22.25	16.25	5	24.6
6	13.0	8.4	6	27.25	20.0	6	28.3
7	15.5	.....	7	32.25	.....	7	32.5
8	18.0	.....	8	37.25	.....	8	37.0
9	20.5	.....	9	42.25	.....	9	43.0
10	23.0	.....				10	49.0
						11	56.0
						12	64.0
						13	74.0
						14	85.0

[a] There were no significant differences in the leaf areas of plants of Early Carman, Delaware, and Up-to-Date that were equally rated.

The estimation of leaf area for one variety, Brownell, showed a bias resulting in ratings for the larger plants that were too high, *i.e.*, the plants were judged to have a greater leaf area than they really had. A separate scale was used for this variety in converting the ratings to areas. In later estimates of leaf area the bias was corrected, and it was possible to use the same scale for all plants.

The rating of November 12 provided a possible explanation for some of the apparent irregularities in previous estimates. It was decided to use a 15-point scale; in fact, the highest rating given was 14. Leaf areas of a number of plants, measured after the estimates of leaf area had been made, were plotted against the ratings for the same plants. From 2 to 14 they formed an evenly ascending series fitting the conception that leaf areas for whole plants were judged on a basis of proportionality, but the mean area of plants rated 1 fell far below what would have been expected from the figures for the larger plants. The scale for November 12 was obtained by fitting a straight line to the points obtained by plotting log leaf area of a number of rated plants against the ratings given them. In class 1 were grouped all plants with a leaf area less than about 12 sq. dm.

Reexamination of the data for the estimates of October 24 and 30 showed that there was an alternative to the arithmetic scale for converting ratings to areas, an alternative that eliminated some of the variability shown in the

measurements of the leaf areas of rated plants. The data were fitted quite well by assuming that the area of plants both above and below 12 sq. dm. was estimated on a basis of proportionality, but that the logarithmic increments between classes for the smaller plants were greater than for the larger plants. Classes 6 to 10 at the third rating (October 24), 3 to 9 at the fourth rating (October 31), and 2 to 14 at the fifth rating (November 12) were in the range where logarithmic increments between classes were relatively small. It is possible that the mind, faced by the problem of rating plants over a relatively enormous range of sizes, reduced the scale for estimating leaf area when the increments in actual leaf area became too large, but continued to make estimates on a basis of proportionality.

TABLE 3.—*Scale for estimating size of potato plants*

Rating	Log mean	Mean	Limits
		sq. dm.	*sq. dm.*
1	0.86	0.7	0.55– 0.95
2	0.10	1.25	0.95– 1.65
3	0.34	2.2	1.65– 2.9
4	0.58	3.8	2.9 – 5.0
5	0.76	5.8	5.0 – 6.6
6	0.88	7.6	6.6 – 8.7
7	1.00	10.0	8.7 – 11.5
8	1.12	13.2	11.5 – 15.1
9	1.24	17.4	15.1 – 20.0
10	1.36	22.9	20.0 – 26.3
11	1.48	30.2	26.3 – 34.7
12	1.57	37.2	34.7 – 39.8
13	1.63	42.7	39.8 – 45.7
14	1.69	49.0	45.7 – 52.5
15	1.75	56.2	52.5 – 60.3
16	1.81	64.6	60.3 – 69.2
17	1.87	74.1	69.2 – 79.4
18	1.93	85.1	79.4 – 91.2
19	1.99	97.7	91.2 –105.0
20	2.05	112.0	105.0 –120.0
21	2.11	129.0	120.0 –138.0

STANDARD METHOD OF RATING PLANTS

It was decided to fix a scale to which all plants rated could be referred, and to design it according to this hypothesis. By using one scale much of the labor and many of the errors likely to result from the use of diverse scales would be avoided. On the other hand, in order to use such a scale plants of known size had to be found and used as standards for every series of ratings made. The scale finally chosen is shown in table 3. It covers a range from 0.7 to 129 sq. dm.; the former represents the leaf area of a young plant almost as soon as the first leaves have expanded above ground and the latter a large mature plant. For plants of a leaf area within the range between $\frac{1}{2}$ sq. dm. to 5 sq. dm. the unit difference between ratings on the logarithmic scale is 0.24, which implies that a unit increase in the rating corresponds to an addition of $\frac{3}{4}$ of the previous leaf area. If this scale were

maintained for bigger plants the increments of leaf area would become too large; so, between the ratings 5 and 11, the logarithmic rate is reduced to 0.12. This means that a unit increase in rating is made for an increase of about one-third in size. For plants of an area greater than 35 sq. dm. the rate of increase for each rating is reduced to about 15 per cent. With very large plants, even this is a large increment.

The usual procedure in rating a block of plants was as follows. The leaf standards, *i.e.*, the prints of the leaves of known standard size, and the scale of sizes for plants were taken into the block of plants to be measured. There was generally an assistant to write down the ratings as the observer gave them. The observer chose one of the biggest plants: from previous experience he might decide it had a leaf area of about 12, according to the rating of the fixed scale. Beginning with the bottom leaf of one shoot, each leaf on the plant was systematically compared with the leaf standards and rated. Immediately the plant was finished, the leaf ratings were converted to areas and the leaf area of the whole plant was found. The rating appropriate to the leaf area was then found from the scale for plants. Instead of 12, the correct rating might be 14. Then one of the smaller plants would be chosen; its rating might be guessed as 4. Perhaps, after measurement, its true leaf area would be found to lie near the border line between ratings 5 and 6 within the range of areas rated 5. Intermediate plants would be measured until the preliminary guesses were found substantially correct. When the leaf areas extended from, say, ratings 2 to 14, perhaps 7 plants would be measured, and intermediate sizes interpolated mentally between those fixed by measurement. Having made a careful examination of the standards, the observer began at one corner of the plot, walking along each row in succession, calling a number for each plant. The assistant wrote this number in the appropriate place on a cyclostyled plan of the plot.

When the rating was complete, a few plants were chosen at random in the plot, their leaf areas measured with the leaf standards, and compared with the ratings already made. The plants originally used as standards also served as checks on the ratings, provided they were well distributed throughout the plot. In a plot of any size, these plants were practically never recognized when the systematic rating of plants was being made. Quite apart from the difficulty of recognizing one particular plant in hundreds the act of rating demanded a degree of concentration that prevented other observations being made at the same time.

ERRORS IN ESTIMATION

The first attempts to use the fixed scale were not altogether successful; but, with practice, the accuracy of the estimations improved beyond that of ratings made before it was devised.

An example of the accuracy attained is illustrated in figure 1, B. These data were obtained from a block of plants set out to see if any differences existed between 25 tuber lines from a commercial stock of the variety Factor

(Up-to-Date). The block contained 600 plants. The leaf areas of 9 plants in various parts of the block were measured to provide standards of reference, the rating of all plants was made, and the leaf areas of 4 more were

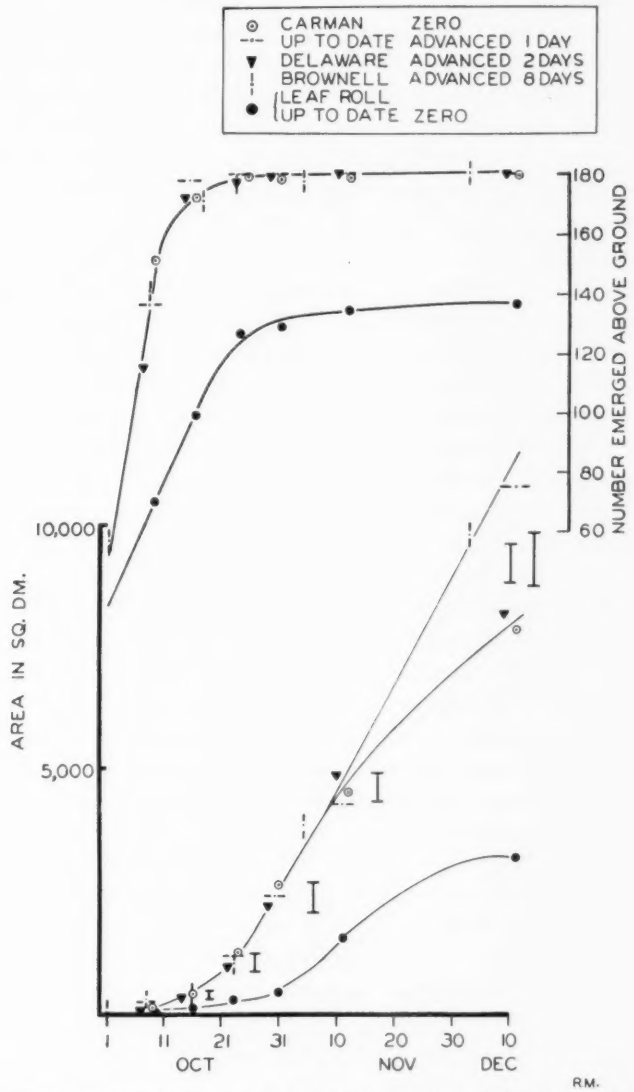

FIG. 2. Emergence and increase of total leaf area for healthy plants of four varieties of potato and one infected with leaf roll. The figures for emergence of the healthy plants were adjusted on the time scale until all varieties fell on the same frequency curve, and equivalent adjustments were made when plotting the leaf areas. Significant differences between leaf areas are shown as vertical lines; for the last rating significant differences between single varieties and paired varieties are both shown.

measured afterwards. The 9 plants first measured were not recognized when they were rated.

The estimates of leaf area for plants measured before and after the rat-

ings were equally reliable. Ten of the 13 plants were correctly rated, the rating of 2 plants was high, and of the other one low. The total leaf area of the 13 plants was, according to the measurements, 129.8 sq. dm., and according to the estimates, 123.1, a difference of 5 per cent. For comparison with these two figures, a third total, 127.2, was obtained by attributing to each plant the mean area for the class into which it fell according to the measurements. This figure is 2 per cent below the total obtained by summing the actual measurements of leaf area. Of the difference of 5 per cent, this 2 per cent may be attributed to errors due to grouping the data, and 3 per cent to errors due to incorrect rating. No bias was introduced into the results by the method of rating, and the breaks in the scale did not affect the accuracy of estimation.

The consistency of rating may be illustrated by an earlier estimate of leaf area from the same experiment. This estimate was obtained before all the plants in the block had emerged from the soil and before the fixed scale was devised. A 6-point scale was used, and the plants barely aboveground or not yet up were rated 0. Two independent ratings were made, one in the morning and one in the afternoon of the same day. About 70 per cent of the plants were given the same rating on each occasion, and all the remainder, except about one per cent, differed by 1 point on the 6-point scale. The combined ratings for unit sub-plots of 4 plants, of which there were 150 in the block, were used in calculating the correlation between the two estimates of leaf area. A value of $r = 0.968$ was obtained, which shows the high degree of association between the two estimates. The mean ratings for the 150 sub-plots were 7.35 and 7.24, *i.e.*, they differed by only 1.5 per cent.

From these results it appears that the method of estimating the leaf area of plants in field plots may be made accurate and reliable, and that the errors of estimation can be reduced below those due to the usual causes of variability affecting the results of field experiments.

APPLICATION OF THE METHOD TO A FIELD EXPERIMENT

A short description of the block of plants mainly used in the development of the method of estimating leaf areas was given earlier in this paper. The leaf area was estimated in order to give a basis for calculating the development of the total aphis population in the block. The results are also of interest as a study in the vegetative growth of several varieties of potatoes and of diseased and healthy plants, although they cover only part of the whole growing period.

Healthy plants consisted of 5 varieties, one of which (probably Great Scot) occurred as rogues amongst another variety (Up-to-Date). As there were no significant differences between them in leaf area over the period when ratings were made, Up-to-Date and Great Scot together are counted as one variety. The diseased plants were of the variety Up-to-Date infected with leaf roll. There were 6 plots in the block of experimental plants, and each plot contained 24 sub-plots, 6 for each variety. There was a leaf roll plant in every sub-plot.

Leaf areas of all plants at 6 different dates were obtained from the ratings, by using appropriate scales, already described. Plot totals for each variety and for the leaf-roll plants were calculated and used as the basis for statistical analysis. The block totals for the different varieties were plotted against time, and the curves were examined to discover what differences existed between them. At first they seemed very different, but part of the difference was obviously due to the late emergence of the variety Brownell of which the seed tubers were not well sprouted at planting. A better comparison was obtained by shifting the curves along the time scale until the lower portions coincided.

To obtain an estimate of the displacement on the time scale for each variety, the number of plants aboveground on the dates when estimates of the leaf area were made were plotted, and curves drawn through them. The most advanced variety was Early Carman: on October 9, 151 of 180 plants had emerged aboveground. Judging by the date on which the curves for the other varieties passed through the point representing the emergence of approximately 150 plants, the variety Up-to-Date (including Great Scot) was one day behind Early Carman, Delaware was 2 days, and Brownell was 8 days. The curve for the leaf roll plants showed that germination was more irregular and spread over a much longer period than for the healthy plants; therefore, no attempt was made to use it as a measure of emergence.

The data for emergence and leaf areas were plotted again (Fig. 2), the 3 varieties other than Early Carman being advanced on the time scale, Up-to-Date by 1 day, Delaware by 2 days, and Brownell by 8 days. There is a close coincidence of the curves for germination. The growth curves also agree until the stage represented by the date November 12 for Early Carman. Later, there is a divergence in the growth of the early varieties, Early Carman and Delaware, from the later varieties, Up-to-Date and Brownell. The rate of expansion of the leaf area of the early varieties decreased very rapidly from the onset of flowering. Flowering began about November 12, when also, presumably, the tubers began to form. The corresponding period for Up-to-Date and Brownell began not long before the last rating was made on December 11.

The only irregularities in the lower section of the curves occur among the readings for November 12, where a smooth curve can barely be fitted to the points within the range of significant differences. This variation probably has little significance, and may merely represent imperfect adjustment on the time scale. The differences on December 12 between early and later varieties are highly significant, whatever small adjustments are made.

The uniformity of growth of the different varieties in the early stages of development is the more surprising, because each curve represents a population of potato plants emerging over a period, and varying considerably in size and vigor. Also, during November and December a number of originally healthy plants exhibited the first signs of infection with leaf roll. To

attain such uniformity it is suggested the following conditions must have been satisfied:

1. The aggregate size of the rudimentary organs of root and shoot must have been of the same order for the populations of healthy plants of all varieties. When the tuber pieces were prepared for planting this plot, they were cut as nearly as possible to a uniform size, and this may have helped produce uniform development of roots and shoots.

2. After the plant emerged, all 4 varieties grew in a similar manner, in spite of morphological differences. Until flowering they behaved as one variety; afterwards, they behaved as two.

3. The differences of leaf area of early and late varieties are due to the incidence of whatever stimulus caused the diversion of metabolic energy from vegetative growth to the formation of flowers and tubers. It did not depend on differences in metabolic efficiency.

4. In the first stages of infection with leaf roll, infection did not greatly affect the growth of the plant.

It is plain from the curves in figure 3 that the emergence and growth of Up-to-Dates were greatly influenced by long established infection with leaf roll. Emergence continued over a longer period, and far less than 100 per cent of the seed tubers produced plants. Growth was slower and less regular than for healthy plants of the same and other varieties. A few comparatively vigorous plants were produced, but, in most, symptoms of rolling and lack of vigor appeared very early. The plants remained stunted for some time. Many appeared to become more vigorous during early November, and this is reflected in the rise of leaf area between October 31 and November 12. The reason for the very slow growth at first appeared to be that internal necrosis and thin sprouts were common amongst the seed tubers. They represented conditions very unfavorable for the transport of food materials to the young plant, and their use in the development of new tissues.

SUMMARY

A method has been evolved whereby the leaf areas of groups of potato plants have been estimated with an accuracy ordinarily assumed to be attainable only by direct measurement.

The estimation is quickly enough made to allow the measurement of comparatively large blocks of potato plants within a few hours.

An example is given of the application of the method to a block of 864 plants laid down as part of an experiment on the transmission of leaf roll. The rates of increase in leaf area of early and late varieties included in the block were similar until the early varieties began to flower; thereafter, they fell below those of the late varieties.

Long-standing infection with leaf roll greatly reduced the rate of increase in leaf area.

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BORON DEFICIENCY IN THE OLIVE

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Because of the relatively high boron content of many California soils and waters (4, 5, 7, 9), it has seemed improbable that any considerable agricultural areas of the State would be found deficient in that element. Thus far only a few small areas have been demonstrated to be affected by such a deficiency. However, a number of soils of the State have been found "inherently low" in boron (12). At any rate, with the number and diversity of disorders to be diagnosed among tree fruits it has become more or less habitual to test all of the well-known minor elements in cases where no good clue is provided by the symptoms. In this way, a considerable area was found in Butte County, California, in which olive trees (*Olea europea*) responded strikingly to borax or boric acid.

HISTORY

In 1917, Horne (6) described an "unusual disease" of the olive and stated "... the disease was called exanthema on account of a certain fancied resemblance to the exanthema or Florida dieback of citrus trees." Horne recognized an association with defects in drainage but noted that excess water did not always produce exanthema symptoms. His description includes withering of tips of shoots resulting in bushy growth, dieback of branches for several feet and puffed bark. The notion persisted for a number of years that these trees were suffering from exanthema chiefly because of resemblance of symptoms to those of deciduous and citrus trees. Smith and Thomas (11), in 1928, stated that olive growers reported beneficial results from copper sulphate. However, Henry P. Everett, County Agent of Butte County, made copper sulphate injections into trunks of affected olive trees in 1938 and 1939, but failed to see any benefit.

In February, 1941, the senior writer, with Mr. Everett, injected olive trees with copper sulphate and also with boron, manganese, and zinc on a small scale in two orchards. The 4 branches receiving boron showed improvement by July, and the contrast became greater as the deficiency symptoms became more pronounced during late summer and fall. The results of these tests and other injections, sprays, and soil treatments made in 1941 and 1942, most of them with the cooperation of Mr. Everett, are reported herein.

DISTRIBUTION OF BORON DEFICIENCY

All of the tests of boron on olive thus far completed were made in the Wyandotte district, Butte County. The olives in this area are grown on Aiken clay loam in rolling foothills. Of the 4,000 acres of olives in Butte County over 3,000 are of the Mission variety. Most of the tests have been

made on this variety but the few trees of the Manzanillo and Barouni varieties, under observation in the district, exhibit essentially the same symptoms. Symptoms are also present in olive trees in the Thermalito district near Oroville, Butte County, and near Loma Rica in adjoining Yuba County.

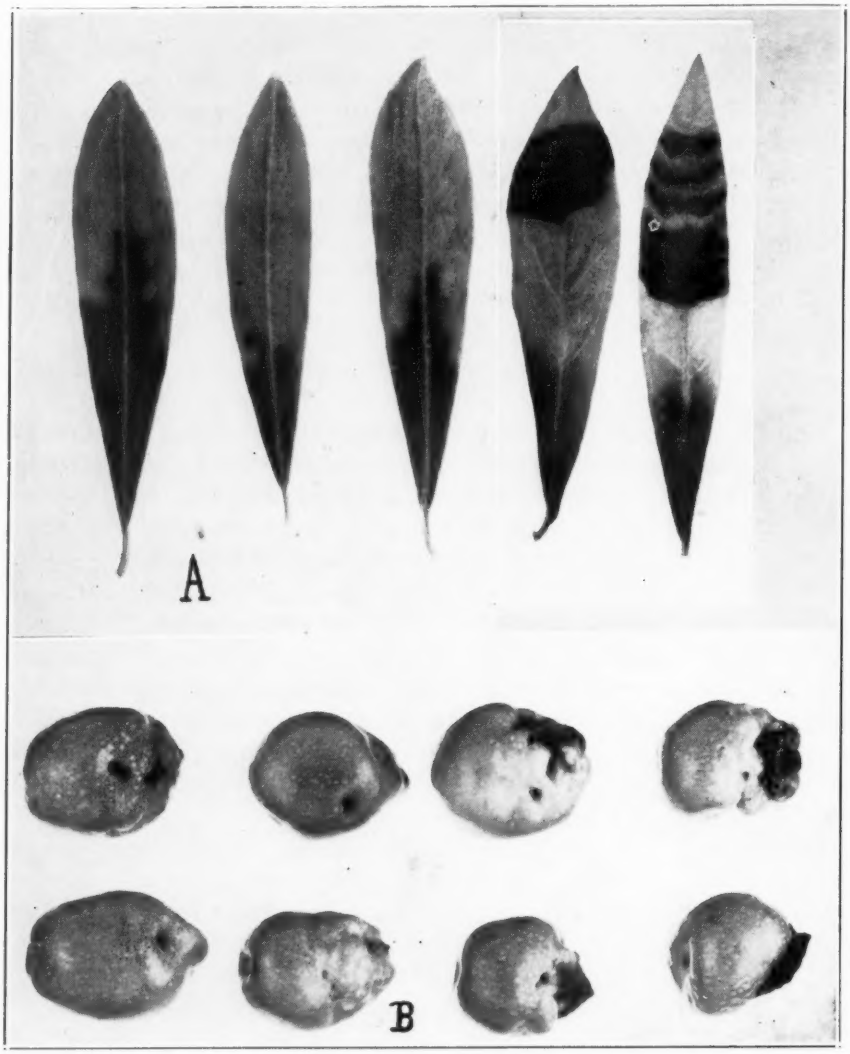

FIG. 1. A. Chlorosis of apical portions of olive leaves and tip necrosis in two leaves at right. B. Pitting and malformation of fruit.

Leaf symptoms indistinguishable from those of trees that respond to boron also have been seen on olive trees in Alameda, Marin, Napa, Sonoma, Santa Clara, and Santa Cruz Counties. In these coastal counties fruit malformation was less common. On the other hand, fruit symptoms but no leaf

symptoms are present in 2 Tulare County orchards in the interior San Joaquin Valley. Boron injection tests have been made in these areas but the results are not yet available. All told, these symptoms have been seen on olive trees growing in a half dozen or more soil types.

SYMPTOMS

Leaf Symptoms

In the Wyandotte district all trees known to be deficient in boron bear some leaves with chlorotic tips (Fig. 1, A). This symptom is quite distinctive, and, since the olive is evergreen and retains its leaves for several seasons, it is present at all times. During fall and winter some leaves of the current season's growth become pale green and some of them eventually bright yellow at the apical end. This chlorosis commonly involves $\frac{1}{3}$ to $\frac{1}{2}$ of the blade, the base remaining normal in color. In rare cases a mere trace of green remains at the base of the blade. This leaf symptom is readily distinguished from the aspect of senile leaves, most of which become yellow all over prior to abscission. Some leaves, which have been chlorotic for some months, develop necrotic tips that bear a zonate pattern (Fig. 1, A). Foliage tends to be sparse on severely affected trees (Fig. 2, B).

Unfruitfulness

Olive trees with mild to moderate leaf and twig dieback symptoms appear to blossom and set fruit normally. Then most of the immature fruits may drop in July and August. The great crop increase on such trees when supplied with adequate boron seems to result mainly from preventing fruit drop rather than from any immediate effect on blossom production. Severely affected trees blossom sparsely.

Defective Fruit

Trees known to be low in boron generally produce some malformed or defective fruit (Fig. 1, B). The pitting, shrivelling, and drying that usually involve only the apical half or less, is locally known as monkey face. All of the flesh under the deep pits and shrunken apical ends is necrotic. Pitting and shrivelling may occur alone or together on the same fruit. On severely malformed fruits pitting also may occur near the stem end. Defective fruit has been seen on the Mission, Manzanillo, and Barouni varieties. Typical counts of fruit of affected trees show a range from 0 to 40 per cent, with individual branches up to 70 per cent.

Dieback and Excess Branching

Severely affected trees present a distinct appearance of short bunchy growth (Fig. 2, A) with only an occasional shoot a foot or more in length. Up to early summer, shoots supplied with adequate boron cannot be distinguished from mildly affected shoots. The first signs of boron deficiency in the latter can be detected in midsummer, when the terminal bud and tip

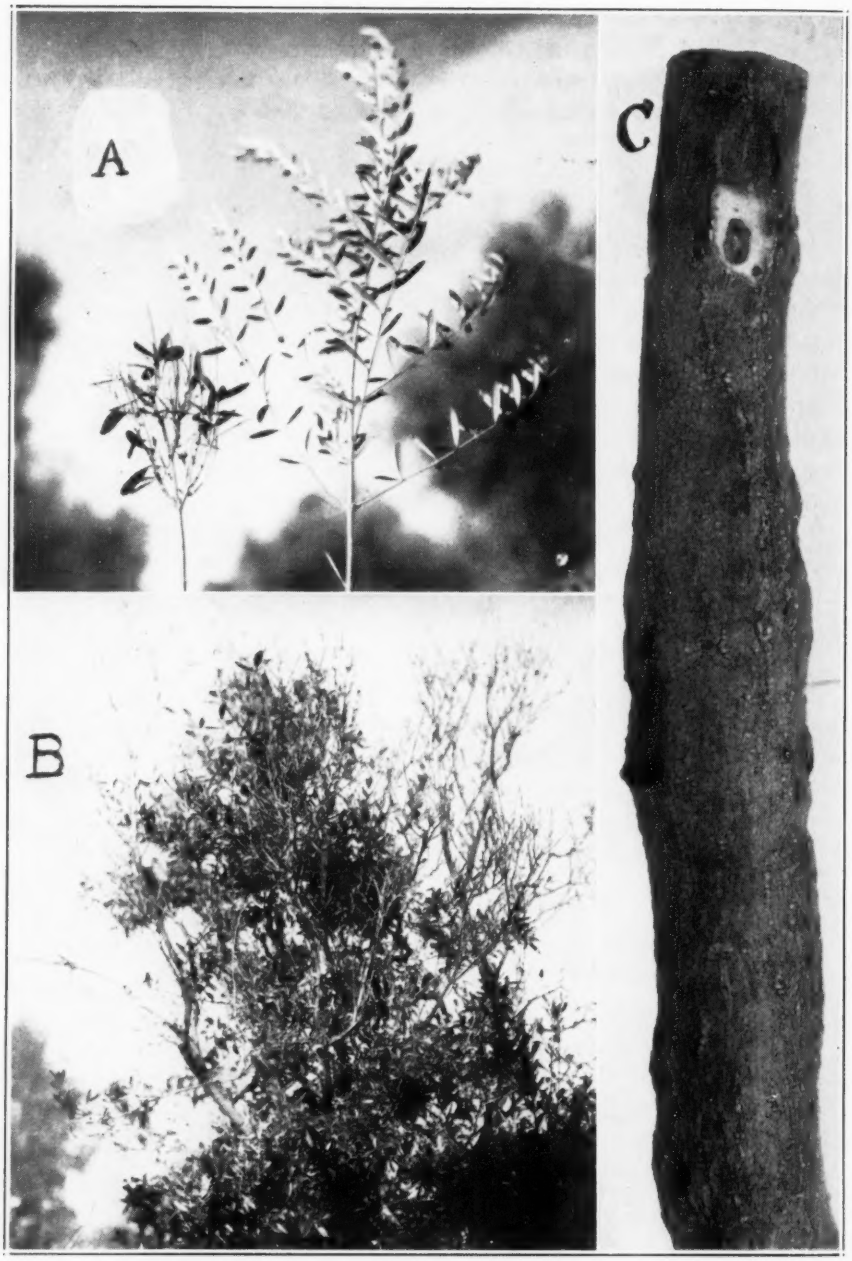

FIG. 2. A. At left a branch severely affected by boron deficiency; at right a normal branch (younger). B. Upper portion of a severely affected small olive tree. C. A branch with proturberances and internal necrosis of bark, ×1.

of the shoot die, usually involving one pair of immature leaves. Two or more secondary shoots are then produced from lower nodes. In severe cases the tips of these secondary shoots die in the same season.

Late in the fall, branches up to an inch or more in diameter die, with leaves persisting. Severe branch dieback has been observed on trees that had been growing fairly well a few weeks earlier and showing but mild leaf and shoot symptoms. Frequently, these branches that die appear to be the most vigorous in the tree. Some trees in the more severely affected areas in the orchards are eventually reduced to mere snags. A branch dieback of unknown cause exists in other olive districts, where boron deficiency is not suspected.

Bark Symptoms

In some of the more severely affected trees the bark of larger branches produces smooth protuberances to perhaps 2 mm. above the level of surrounding bark and mostly 5 to 10 mm. long (Fig. 2, C). The bark underlying these protuberances contains varying amounts of brown necrotic tissue. On the same branches or elsewhere the bark may crack and become considerably roughened. As in the case of boron-deficient apples, however, the bark symptoms are not consistently associated with those of fruit and leaves.

METHODS

The dry-salt method was employed in all limb injections of copper sulphate, manganese sulphate, zinc oxide, and boric acid. The powdered boric acid was mixed with an equal weight of diatomaceous earth (Celite) to facilitate measuring and handling.

A "gun" having the same dimensions as that described by Bennett (3) was used as a convenient instrument for measuring and inserting the dry salts. The boric acid-Celite mixture contained approximately $\frac{1}{4}$ g. of boric acid per shot. Dosages ranged from $\frac{1}{2}$ to 2 shots to each hole. One hole was used in branches 2 to 3″ in diameter and usually two holes in branches 4 to 6″ in diameter. The holes were 2″ or more in depth, bored with a 15/32-inch wood drill and plugged with $\frac{1}{2}$-inch hardwood dowels. Bark injury was reduced by dipping the ends of the dowels in soft wax before inserting.

Borax was applied with an orchard sprayer at 400 lb. pressure. A proprietary detergent (Dreft) was added to the June sprays to facilitate leaf wetting. The upper surfaces were wetted satisfactorily but the deposit on the under surfaces was scanty.

In the only soil treatment made prior to the summer of 1942 borax was spread by hand in bands around the trees, extending approximately from 2 to 10 feet from the trunk. The soil was not cultivated for several months after the application. About 10 inches of rain fell between the time of application and the summer dry season of 1942. Later soil treatments were made broadcast in irrigation furrows and in one case in solution applied from a spray tank.

RESULTS

Branch injections of boric acid, copper sulphate, manganese sulphate, and zinc oxide were made in Mission olive trees in 2 orchards in February, 1941. The trees were selected chiefly on the basis of tip dieback as described above. By July each of the 4 branches treated with boric acid showed definite improvement in growth when compared with the balance of the tree. From midsummer on, most of the terminal buds on nontreated portions of these trees died, while treated branches continued to grow vigorously. Two of the 4 branches treated with boric acid bore a heavy crop, while there was very little fruit on the nontreated branches. These treated branches also continued to grow normally throughout the second season. Additional branch treatments were made with copper, zinc, boron, and magnesium in November, 1941. No certain effect from any of these treatments could be seen in mid-June, 1942. By August, however, all of the boron-treated branches showed recovery and contrasted strikingly with the untreated portions and with branches treated with the other elements.

Five trees showing good vegetative growth but a high incidence of malformed fruit were treated in November, 1941. Each of the 5 trees was injected with boron, copper, manganese, and zinc, each in separate branches. In these trees the boron-treated branches bore more fruit in 1942 than the total produced on the balance of the tree. In addition to the increase in crop there was a sharp reduction in the amount of defective fruit on the boric acid-treated limbs (Table 1). No clear effect of copper, manganese, or zinc has been seen up to February, 1943.

TABLE 1.—*Effect of branch treatments on defective fruit of Mission olive. Hays Orchard, Butte County. Treated: November, 1941. Counts made: October, 1942*

	Branch treatment				
	None	Boron	Copper	Manganese	Zinc
Total fruit counted	446	700.0	200	100	169
Defective fruit	174	17.0	70	27	64
Percentage defective fruit	39	2.4	35	27	37

Although boron seems to move laterally and downward more readily than some of the other minor elements, such movement is definitely limited. Thus, when branches 4 to 6 inches in diameter are injected in a single hole, the benefit is greater on that side of the branch than on the opposite side, at least so in the first season after treatment. Branches a foot below and in line with the point of injection are improved during the first season but not completely cured.

Sprays

Mission olive trees were sprayed in March, 1942, with borax, 2 and 6 lb. per 100 gal.; manganese sulphate, 10 lb. per 100 gal.; 8–4–50 Bordeaux mixture and red copper oxide, 3 lb. per 100 gal. Some benefit was apparent

in growth, set, and quality of fruit on the trees sprayed with the 2 lb. dosage of borax, but the improvement was much more certain with the higher dosage. Copper and manganese were not effective. The March sprays were subjected to about 10″ of rain.

Additional spray applications were made on June 11, when most of the trees had just completed the blossom stage, but a few branches were still in full bloom. No rain fell during June, July, and August. The crop was markedly increased on most of the trees sprayed at this time in contrast to trees in nonsprayed rows, which continued to drop fruit throughout the season. Four trees severely stunted by boron deficiency, included in the 8 lb. to 100 gallon plot, failed to set any fruit, and were not greatly benefited in growth. Fruit counts on 10 nonsprayed trees in this orchard gave an average of 13.2 per cent defective fruit. Twenty-seven trees sprayed with 2, 4, or 8 lb. of borax to 100 gallons in March or June produced a much larger crop and only 0.41 per cent defective fruit. No significant difference was detected between trees sprayed in March or June.

June spray tests in a second orchard afforded less definite results, perhaps because of a mixture of varieties. In the same orchard one side of each of 6 severely affected Mission olives was sprayed with borax 8 lb. to 100 gallons on June 11. Improved twig growth was noted in July on the sprayed sides, but the benefit had disappeared by October. In a third orchard trees sprayed on June 11 seemed improved throughout the season.

Soil Treatments

In February, 1942, Mr. Everett applied borax on the soil around 11 large trees in amounts ranging from 3 to 32 ounces per tree. All of these trees showed benefit in shoot growth by midsummer and good shoot growth continued through the season on trees that received 6 ounces or more. Some defective fruit (0.3 per cent to 9 per cent) was found at the 6-ounce dosage, but only one branch bore such fruit on the 4 trees that received 13 ounces or more. The 1942 crop was increased very considerably by the heavier dosages.

Borax treatments of ½ to 4 pounds per tree were made in 2 orchards in August, 1942. The material was lightly covered with soil in the bottom of the irrigation furrow shortly before the application of water. Clear benefit to shoots and leaves was apparent in one of these orchards in February, 1943. No evidence of boron toxicity to olive trees has been seen thus far in any of the soil treatments.

Boron Deficiency in Other Crops in California

Unthrifty orange trees adjoining affected olive trees have been under observation in Butte County. Definite signs of boron deficiency were not detected in these trees, although the fruit seemed unnaturally hard and was subject to abnormal cracking at the blossom end. In June, four orange trees were irrigated in small basins with a solution of borax, supplying about ½ lb.

to each. Symptoms of excess boron developed in the leaves by August and were more evident in October. New growth was appearing at this time, but there was no clear benefit to growth as compared with that of neighboring trees during the current season. Young peach trees adjoining those of citrus and olive were not visibly benefited by a borax spray (2 lb.–100 gal.) applied in June.

Borax heretofore has been used in California as a soil amendment to a very limited extent, although deficiency symptoms in widely separated areas have been reported. Ark and Thomas (1) found that heavy soil applications benefited apple trees in Sonoma County. Corky fruit was found in one apple orchard in Eldorado County, and was corrected by trunk injections by Thomas in 1938. Larmer (8) described symptoms believed to indicate boron deficiency in sugar beets in the Salinas Valley but no field tests seem to have been made. C. M. Tompkins (unpublished) has seen boron deficiency symptoms in celery, radish, lettuce, and table beets in light, well-drained soils at Colma in San Mateo County and nearby in San Francisco County. Borax, broadcast at the rate of 20 lb. per acre and disked in just prior to seeding, eliminated symptoms in subsequent crops. O. A. Lorenz (unpublished) has observed the boron deficiency type of symptoms in table beets in Alameda, Monterey, Sonoma and San Joaquin Counties and has verified his diagnosis by pot cultures for the Sonoma soil. John T. Middleton (unpublished) observed symptoms attributed to lack of boron, on broccoli, cabbage, cauliflower, and rutabaga at San Juan Capistrano in 1940 and 1941. The symptoms usually were associated with limestone outcroppings where the soil pH was higher than in surrounding soil where plants were normal. Borax alone, applied for two seasons, failed to correct the symptoms; but, after sulphur treatment in the third season the crops were normal in these areas.

Ark and Tompkins (2) found that a borax solution eliminated symptoms in greenhouse Gloxinias. The symptoms developed with Gloxinias grown in "ratsnest," a mixture of forest débris collected in Marin and other coastal counties.

Trunk injections of boric acid made by the writers seemed to benefit Vinifera grapes in one Sonoma County vineyard. Low boron in the soil is indicated by the presence of pronounced leaf symptoms in olive near the vineyard. Attention was called to this vineyard because of defoliation and fruit shrivelling or failure to mature in 1941. The work on grapes has not progressed far enough to clearly define local symptoms, but those observed agree fairly well with the symptoms reported elsewhere (4, 10).

DISCUSSION

The severely affected olive trees that attracted the particular attention of growers usually were in small groups in association with impaired drainage or excessive seepage of ground or irrigation water. With the discovery of boron deficiency and the leaf and fruit symptoms it became evident that the trouble was more extensive in the district than previously realized. The

tests to date have been confined to trees showing specific leaf or fruit symptoms or both. Extensive use of borax by olive growers during the winter of 1942–43 should show whether trees that do not exhibit the described symptoms will respond in growth or fruit production.

The olive is notoriously irregular in yield from season to season, tree to tree, and even from branch to branch in the same tree. However, a striking increase in crop from some of the treatments was obvious at a glance. Indeed considerable difficulty was often experienced in finding enough fruit on nontreated trees or branches to permit counts of percentages defective. Rather extensive yield records will be required nevertheless to determine the magnitude of crop increase.

The striking symptoms on the olive, some of which persist the year round, and the widespread planting of olive trees at roadsides and in gardens suggests that this tree may become a useful indicator of boron deficiency in areas not devoted to the commercial production of olives. Preliminary surveys seem to bear out this suggestion.

As to boron injury, Eaton (4) places the olive about midway in the semitolerant group. With the possible exception of one limb injection, no injury has been observed from any of the tests made by the writers. Soil application of a maximum of 8 lb. per tree are under way. Since large olive trees are improved by as little as 3 and 6 oz. of borax per tree and orange trees injured by 8 oz. per tree, it is obvious that the fixing power of the Aiken soil for boron is not high.

On the basis of the limited trials with olives and of experience elsewhere with deciduous fruit trees, commercial treatments have generally been at the rate of 1 lb. per tree or 50 lb. per acre. In some cases this dosage has been doubled in either a single application or by a second application on more severely affected areas. Fifty tons of borax for application during the winter of 1942–43 has been delivered to the growers of the Wyandotte district.

SUMMARY

Characteristic pitting of fruits, chlorosis of leaf tips, and dieback of shoots and branches of the olive were reduced or eliminated by treatment with borax or boric acid. Branch injections, soil treatment, and spraying with boron compounds were all beneficial; but the effect of spraying on severely affected trees was transitory. Large trees responded to less than ½ lb. of borax broadcast on the soil in one district of Butte County, but seem to require about 1 lb. for complete cure. Four pounds per tree in the same soil did not cause discernible injury up to 6 months from the time of application.

Observations and tests of boron deficiency in other crops in California are summarized.

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THE INHERITANCE OF A WHITE MUTANT CHARACTER IN USTILAGO ZEAE[1]

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Since Bauch[2] first reported the occurrence of sector variants in cultures of haploid lines of *Ustilago bromivora* (Tul.) Fish. v. Waldh., the phenomenon has been investigated extensively in several smut fungi, especially *U. zeae* (Beck.) Ung. Several investigators have shown that sector variants appear commonly in cultures of monosporidial lines of *U. zeae*. Stakman, Christensen, *et al.*[3] made an extensive study of variation in this fungus. They pointed out that numerous variants were produced in some monosporidial haploid lines but not in others, and that the tendency to sector could be increased in certain lines by certain environmental conditions. There were some indications from their investigations that factors for variant characters were inherited when mutants were crossed with other lines. All of the evidence available indicated that variants resulted from genetic changes and probably could not be attributed to delayed segregation but rather to mutation.

Stakman, Tyler, and Hafstad[4] studied the constancy of cultural characters of a number of variants of *Ustilago zeae* and confirmed the previously expressed opinion that variant lines retained their distinctive characters, although subject to pronounced phenotypic variability. In addition, they studied the pathogenicity of a haploid monosporidial line and several of its variant derivatives by injecting them into corn, each in separate combinations with two haploid monosporidial lines of different sex. They concluded that variants in general tended to be less virulent than their parents, but that an occasional variant might be somewhat more pathogenic. As the resulting dikaryophytes differed distinctly in pathogenicity, it was evident that the variants were different genetically from the parental lines. Furthermore, the pathogenicity of the various combinations was the same in tests made about 3½ years apart, thus again showing persistence of variant characters. The conclusion regarding genotypic differences between the parental line and its variants was based on relative behavior in the dikaryo-

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[2] Bauch, R. Untersuchungen über die Entwickelungsgeschichte und Sexual-physiologie der *Ustilago bromivora* and *Ustilago grandis*. Ztschr. Bot. **17**: 129–177. 1925.

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FIG. 1. Origin of mutant and inheritance of mutant characters. 10K2 is the brown line, characterized by radial folds, from which the white mutant, with concentric rings, 10K2–1, arose. Colonies from the four sporidia from each of three chlamydospores, F,

phase; but it was not shown that the variant characters were transmitted to f_1 segregates, the haploid progeny of crosses between haploid lines. Therefore, further work was done along this line, and conclusive evidence was obtained that factors for variant characters are inherited. Stakman[5] presented evidence that factors for mutability are inherited just as are factors for cultural characters.

During the course of extensive experiments on variation, according to evidence presented briefly by Stakman *et al.*,[3] it was found that delayed segregation could scarcely account for the production of sector variants. It was quite clear that a culture derived from a single sporidium constituted a pure line, barring subsequent mutation.

Attempts were made to find a mutant character so distinct as to enable the easiest possible recognition in segregates from crosses. This seemed particularly important because there is such extensive recombination in many crosses that even the parental types sometimes are not recovered among the f_1 segregates. Several years ago a mutant of the desired type appeared, and it has been studied since that time. As only some of the results are pertinent to the present discussion, a relatively brief summary is presented here. Further details are given in a paper on the inheritance of factors for constancy and for variability in *Ustilago zeae*.[6]

In 1934, an apparently pure white sector (designated 10K2–1) appeared in a colony of 10K2, a monosporidial line that always produced brownish colonies tinged with vinaceous. This white sector contrasted so strongly with the colony as a whole that transfers were made from it, and the resulting cultures were compared in detail with the parent. It was evident that most of the factors for pigmentation had been lost as there was only a tinge of color in the mutant under some conditions, whereas the parent culture in which the mutant arose was always brownish. In addition to loss of factors for pigmentation, the mutant had a tendency to produce abundant aerial conidia, which accentuated the impression of whiteness. In this respect the mutant also differed decidedly from the parent 10K2.

The mutant differed from its parent in a third character, namely, the tendency to produce numerous narrow, concentric rings, whereas the parental line had a tendency to produce more or less distinct radial furrows (Fig. 1). It was evident, therefore, that the mutant, designated 10K2–1, differed from its parent 10K2 in three distinct characters. Both the parent and the mutant were then crossed with a monosporidial line 10I1. This line is shown in figure 1, and a brief description of it is given in table 1. All three mutant characters appeared in various combinations in the f_1

[5] Stakman, E. C. The problem of specialization and variation in phytopathogenic fungi. Genetica 18: 372–389. 1936.

[6] Stakman, E. C., M. F. Kernkamp, T. H. King, and W. J. Martin. Genetic factors for mutability and mutant characters in *Ustilago Zeae*. American Journal of Botany 30: 37–48. 1943.

M, and K, resulting from the cross between 10K2–1 and 10I1 are shown below. The white color of the mutant appears in the segregates from each clamydospore. Of the five white lines, three (F1, F3, and K3) are sectoring.

progeny of the cross of 10K2–1 × 10I1. There were no white segregates, and none of the other mutant characters appeared in the progeny of the cross of the 10K2 (parent) × 10I1.

TABLE 1.—*Cultural characters of monosporidial lines 10I1, 10K2, and mutant from 10K2, 10K2–1.*

Lines	Color	Topography	Margin
10I1	Clear brown to tan, tinged with purple	Close pronounced radial folds tapering toward periphery	Clear, somewhat ciliate
10K2	Warm buff to brown, with vinaceous tinge	Fine radial folds; confused clockwise folds in center; counter-clockwise folds towards margin	Clear, somewhat ciliate
10K2–1	White	Umbonate; with distinct concentric ridges	Silky, ciliate

For the sake of clearness, only the inheritance of factors for "white" color is emphasized in this paper. It should be pointed out that there are varying degrees of whiteness, but segregates were recovered that were as white as the mutant. The impression of whiteness is given by the relative absence of pigment in the compact mycelial growth of colonies plus the rather abundant development of aerial conidia on the surface. Figure 1 shows single colonies of 3 sets of sporidia isolated from 3 promycelia from chlamydospores resulting from crossing the mutant 10K2–1 with 10I1. It will be noticed that 5 of the segregates are distinctly white in appearance, but none of them is exactly like the mutant parent and only 2 of them are alike. There had, therefore, been a certain amount of recombination, but the most conspicuous feature common to all of them is their whiteness. It will be observed also from figure 1 that three of the white segregates, F1, F3, and K3, are producing sectors different in appearance from the rest of the colony. Many such sectors were studied and proved to be distinct.

From cross 14 (10K2–1 × 10I1), 10 out of 38 f_1 segregates were white. The same cross was made again and 9 out of 28 segregates were white, making a total for this cross of 19 white segregates out of 66 that were studied.

One of the white segregates from cross 14, designated 14J4, was then crossed with 26G1–2, a line that was as nearly black in culture as any that the writers have studied. This cross was designated cross 300. Of 32 segregates from this cross, 18 were white or near white; single colonies of sets of four sporidia from the promycelia of each of three chlamydospores are shown in figure 2.

Several other white lines from cross 14 were crossed with other dark-colored lines, and in every case white segregates appeared.

At present there are 83 distinct white or near white derivatives from the original white mutant, all of which arose as segregates from crosses involving progeny of the original mutant.

As it was apparent that the white mutant character was due to genetic

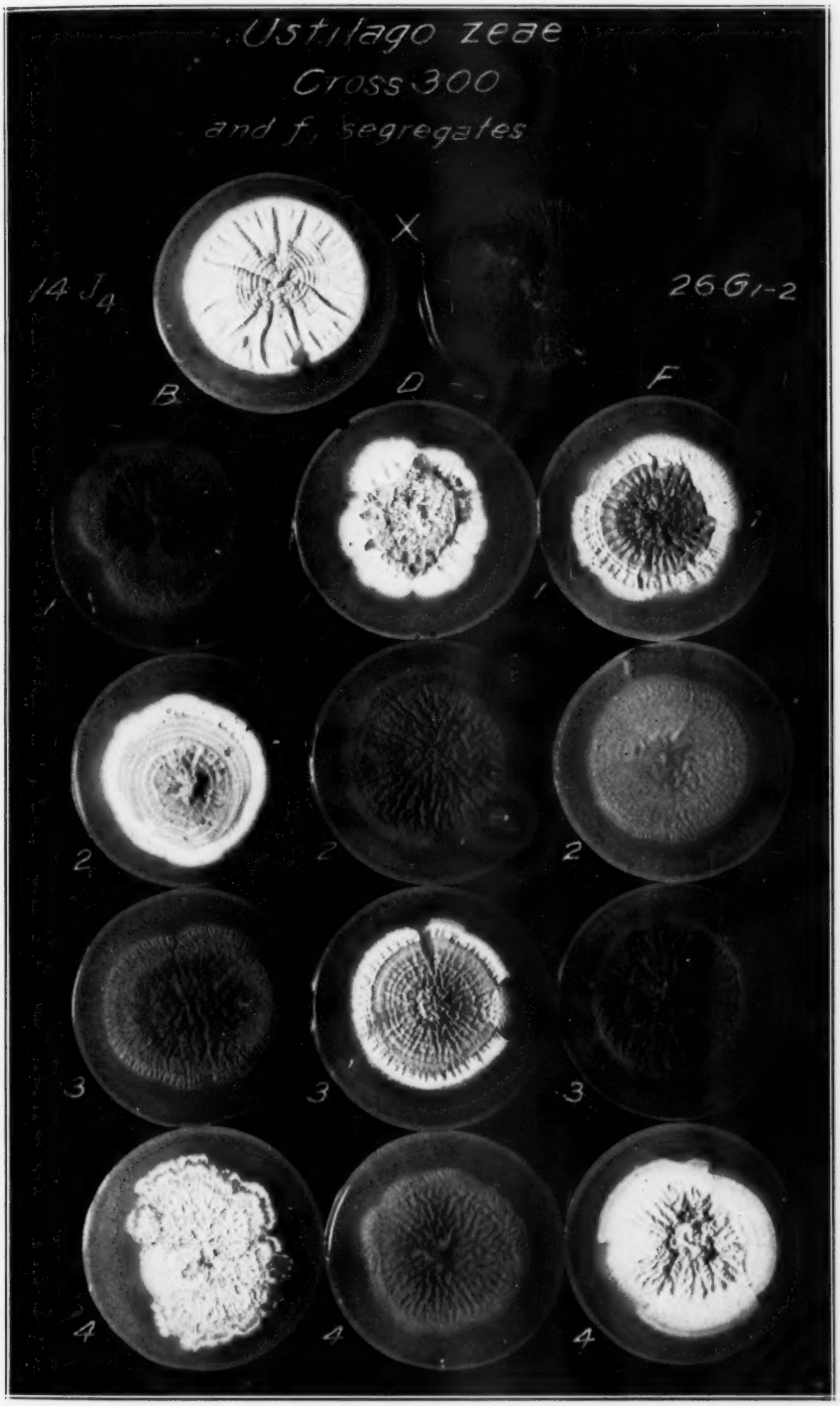

FIG. 2. Single colonies from the four sporidia of the promycelia of three chlamydospores, B, D, and F, from the cross between the white line, 14J4, and the black line, 26G1–2. The white color appears among the segregates from each chlamydospore.

factors that were inherited in crosses, there can be no question that genetic changes were involved and that the genes for this character were inherited in the same way as those for other characters. It seemed possible, therefore, that a white smut might be produced by appropriate crossings of white × white.

Thirty-nine of the whitest lines available were mated in various combinations, making a total of 417 matings, and inoculated into corn seedlings, both in the greenhouse and in the field. Many of the compatible combinations produced large galls on the corn plants, indicating that the compatible lines were of opposite sex and contained the necessary factors for pathogenicity and gall formation. Surprisingly enough, however, there were no mature chlamydospores in any of the galls and only in a few galls were there apparently immature chlamydospores. After repeated attempts to obtain combinations that would produce chlamydospores, all of the white lines were mixed together and inoculated into corn. Large galls were formed, but there never were any mature chlamydospores. These trials were repeated several times, but the results were always the same.

It appears, therefore, that the white mutant had lost certain factors for ability to fuse with lines of its own kind. The necessary factors for nuclear association and the production of the dikaryophase are present; furthermore, the necessary factors for pathogenicity obviously are present in many of the dikaryophytes, since they produced normally and, in some cases, even extraordinarily large galls. Nevertheless, the necessary factors for nuclear fusion and the production of zygotes, the chlamydospores, apparently are lacking. That these white lines are not all of the same sex is indicated by the fact that only certain combinations among them will produce galls while others will not, and furthermore by the fact that when white lines are crossed with a random sample of tester lines, they assort themselves into various sex groups, which produce normal chlamydospores as is true of a random sample of lines in general.

Finally, corn was inoculated with a mixture of 12 white monosporidial lines derived from haploid segregates from 5 different crosses. Galls were formed but they contained no chlamydospores nor chlamydospore-like bodies. Material from some of these galls was killed in formal-acetic alcohol, run through the butyl-alcohol series, embedded, cut about 8 microns thick, and stained by the Feulgen method. The mycelium was as clearly dicaryotic as in normal galls. Hence it appears that certain combinations of the white lines under consideration produce a normal dicaryophase, without being able to produce the diplophase.

SUMMARY AND CONCLUSIONS

From the foregoing it is evident that factors for the white mutant characters that arose in a brown colony are heritable through several successive sexual generations. This should furnish final proof that such sector variants are mutants. That a genetic change had taken place is perfectly

obvious, and that the new characters, are persistent both in asexual propagation and sexual reproduction is also clear. One of the most significant facts, however, is that it never has been possible to obtain functional chlamydospores from crosses between the white derivatives of the original white mutant. In a sense this indicates that there are multiple factors for sex. The nuclei of certain white lines have the necessary factors for attraction and association that enable them to produce the dikaryophase, but they seem to lack the necessary factors for complete sexual fusion. This experience and many others of similar nature suggest strongly that there are gradations in the degree of maleness or femaleness, if these terms could be applied to an organism that is completely isogamous as far as can be observed. It is interesting also to note that factors for pathogenicity are apparently different from those for sex. It is true that *Ustilago zeae* is pathogenic only in the dikaryophase, or, in exceptional cases, in the diplophase; consequently, the association of nuclei of two kinds is prerequisite to parasitism in the corn plant. But these two nuclei are not necessarily able to fuse and produce functional zygotes or chlamydospores.

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THE NATURE OF ULTRA-VIRUSES AND THEIR BIOLOGICAL ACTIVITY[1]

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Half a century ago D. I. Ivanovsky (5) discovered ultra-viruses. At the dawn of virulogical investigations two points of view concerning the nature of viruses were advanced. According to the conception of Beyerinck (2), ultra-viruses differ from microbes in principle (*Contagium vivum fluidum*); according to Loeffler and Frosch (6), they are simply very minute microbes. The isolation by Stanley (16) of the purified virus of tobacco mosaic showed that the direction of virulogical research pointed out by Beyerinck is a more fruitful one. Thanks to the discovery of virus nucleoproteids and their subsequent physico-chemical analysis, a magnificent picture opened before our eyes. Biological individuals are to be found in the world of molecules, subject to the laws of microphysics. From a nude protein molecule to a real microbe there exists a whole series of intergradations. This series includes elementary bodies (about which the discussion still lasts whether they are molecules or protoplasts separated from the surrounding world by semipermeable membranes), real ultra-microbes leading the life of parasites, and saprophytic ultra-microbes. At present we do not know whether this series is an ascending or descending one. It is also certain that to a definite degree it is an artificial series. Nevertheless, it obliterates the border line between molecules and protoplasts, a most remarkable regularity is observed in this series, which can be formulated thus: the simplification of the organization of the cell and its conversion into precellular condition is connected with the concentration of nucleoproteids. The cells of bacteria are richer in nucleoproteids than those of higher organisms, elementary bodies are richer in nucleoproteids than the cells of bacteria, viruses pathogenic for plants are pure nucleoproteids. The statement that nucleoproteids form the substratum of continuity and variability of the most elementary biological units is firmly established through virulogical investigations.

Up to recent times viruses were studied chiefly from the physical and chemical points of view. Notwithstanding the importance of these studies, they cannot answer the most fundamental questions concerning the mechanism of autoreproduction of virus molecules and the mechanism of their action. To the study of these questions the works of the writer and his collaborators have been devoted.[2] Though the results obtained are far from giving an exhaustive answer to these questions, they nevertheless include a certain program of research, and plan concrete ways of experimental approach to these difficult questions.

[1] Report made at the Session of the Academy of Sciences of the Ukrainian S. S. R. (12th–17th Jan., 1942).

[2] Institute of Microbiology, Academy of Sciences of the U. S. S. R.

At the beginning of our investigations we made an attempt to find out what the virus takes from the plant by means of fractioning the proteins of the diseased plant. We failed to detect a deficit in the protein fraction, which coagulates at 70° C. in the juice of tomatoes affected by mosaic disease; on the contrary, we got the impression that, alongside the virus protein in the diseased plant, there can accumulate an inert non-virus protein—a phenomenon already pointed out by other authors (11).

If diseased and normal plants are grown under conditions of phosphorus and nitrogen starvation, the diseased plants lose less protein than the normal ones and, consequently, the difference in the contents of protein nitrogen between diseased and normal plants is more pronounced in starving plants than in those that obtain normal nutrition (Table 1).

TABLE 1.—*Contents of protein N in leaves of tobacco under different nutrition (in per cent of dry weight)*

Nutrition	Normal	Diseased	In per cent of control
NPK	2.06	3.61	172.9
0.25N/PK	1.05	2.54	240.5
N/0.25 P/K	1.62	3.44	212.8

Some literature data (4), as well as other works of the writer, completely affirm our data concerning a more economical utilization of protein by plants affected by virus diseases.

The investigation of nitrogen metabolism does not indicate what is taken from the plant by the virus; an exception is found only in the data of our collaborator, P. A. Agatov (1), who showed that the proteins of tomatoes affected by the mosaic disease contain somewhat less argynine and proline than the proteins of a normal plant. Failing to find in the nitrogen metabolism an answer to the question investigated, we turned to the study of phosphorus metabolism in case of virus diseases. It was found that tobacco infected by the mosaic disease contains no more (and often less) phosphorus than a normal plant. The lipoid phosphorus undergoes no significant changes, whereas the changes in protein phosphorus are rather clearly manifest. The amount of phosphorus in soluble proteins in case of mosaic disease increases, the amount of phosphorus in insoluble structural protein de-

TABLE 2.—*Forms of P in leaves of mosaic-diseased and normal tobacco (in percentage P_2O_5 of dry weight)*

Material	Total	Mineral P and phytine	Organic P	Protein-soluble P	Protein-insoluble P
Normal	1.24	0.59	0.30	0.067	0.34
Diseased	1.18	0.54	0.34	0.198	0.28
Normal	1.23	0.51	0.28[a]	.....	0.44
Diseased	1.24	0.57	0.39[a]	.....	0.40

[a] Including protein soluble.

creases. It seems that in case of virus diseases there takes place an expenditure of structural nucleoproteids (Tables 2 and 3).

TABLE 3.—*Forms of phosphorus in leaves of mosaic-diseased and normal tobacco (in percentage dry weight)*

Material	Total	Lipoid	Soluble in 0.1 N HCl	Insoluble
Normal	2.25	0.19	1.54	0.62
Diseased	1.89	0.16	1.34	0.56

A comparison of normal and diseased plants is insufficient for a characteristic of biochemical changes that are connected with the accumulation of the virus, because the picture is evidently strongly marred by diverse secondary factors. In order to eliminate as far as possible these secondary factors we compared leaves rubbed with the juice of a normal tobacco plant with those rubbed with the juice of a tobacco plant infected by mosaic, or compared isolated halves of leaves likewise rubbed with normal juice or with juice containing the virus.

Thus, leaves accumulating and not accumulating the virus were compared. Externally, they differed but insignificantly, those accumulating the virus being on the average somewhat yellower than normal ones. The data presented in table 4 show that the accumulation of the virus is accompanied by an expenditure of carbohydrates; while normal leaf halves have spent a considerable amount of protein, leaf halves accumulating the virus have scarcely spent protein at all.

TABLE 4.—*Carbohydrates and nitrogen in normal tobacco leaves and in leaves accumulating the virus (in percentage dry weight)*

Material	Glucose	Saccharose	Starch	Protein N	Total N
Leaves rubbed with normal juice	0.85	0.71	1.50	3.89	5.93
Leaves rubbed with infected juice	0.49	0.28	0.26	4.07	6.05
Isolated leaf halves rubbed with normal juice	0.15	0.35	0.61	3.32	6.08
Isolated leaf halves rubbed with infected juice	0.11	0.23	0.04	4.03	5.76

We further sought in the possible biochemical activity of the virus an explanation of its powerful action on the metabolism of the plant. The first tests of the purified virus of tobacco mosaic in respect to its possible fermentative activity gave negative results (8). However, works in this direction were continued in our laboratory. M. N. Vorobiova (18) observed that the protein of the virus of tobacco mosaic, when activated by CN or HS, can hydrolize the tomato protein. In cooperation with this author we were able

to detect the ability of this virus to liberate phosphoric acid from monophosphate of glucose (Table 5).

These data as to the fermentative activity of filtrable viruses agree with some literature evidence, especially concerning bacteriophage and ele-

TABLE 5.—*Action of the phosphatase of the virus of tobacco mosaic disease*[a] *(pH 5.6, temperature 37° C., substratum-monophosphate of glucose)*

Mineral P in percentage		Percentage of control	Exposition in hours
Control	Experiment		
0.16	0.19	120.2	2
0.15	0.22	147.9	18
0.13	0.22	174.5	18
0.24	0.55	225.8	20
0.15	0.40	257.5	18

[a] Note: in all the experiments except the second one magnesium sulphate was added to obtain the activation of phosphatase.

mentary bodies of vaccine, but require further verification and expansion. Seemingly, viruses actually have the action of phosphatase and one of the primary links of their biochemical activity is formed by phosphorilizing of carbohydrates and therefore the activation of carbohydrate metabolism, as well as the disintegration of structural nucleoproteids of the protoplast infected by the virus.

We also sought the elucidation of the relation between the protoplasm and the virus in experiments concerning the influence of starvation on the titre of the virus. In these works fulfilled partly in co-operation with Smirnova (9), partly by Smirnova alone (13), we failed to detect a decrease of the titre of the virus in plants deprived of nitrogen or phosphorus. Spencer agrees with us that the virus protein, when once formed, is not destroyed in starving plants; however, this author thinks that in tobacco plants deprived of nitrogen the rate of accumulation of the virus is slackened. The discrepancy of our results probably depends in a large degree upon the fact that Spencer freezes the plants whence he draws the juice for the determination of the titre of the virus. This procedure is connected with a partial inactivation of the virus, and our preliminary data show that this inactivation is accelerated in plants deprived of nitrogen.

An investigation of the mechanism of the accumulation of the virus is impossible without a thorough knowledge of the physiological conditions necessary to such an accumulation. Strangely enough, data on this question are nearly absent in literature. Here we applied the leaf halves method. Tobacco leaves separated from the plant were rubbed with the juice containing the virus, which was subsequently thoroughly washed from the surface under a water tap. The leaves were cut in halves, each experimental half corresponding to a control one. The experimental halves were exposed to the action of different temperatures, were placed in an atmosphere of pure hydrogen or were infiltrated with different substances.

By this means we were able to ascertain that the accumulation of the virus of tobacco mosaic disease is already absent at a temperature of 5° C., and proceeds much more slowly at 15° C. than at 20° C. (Table 6). The temperature curve of the accumulation of the virus is to be given later on (14). The virus continues to accumulate in an atmosphere containing 0.5 cc. of

TABLE 6.—*The influence of temperature on the titre of the virus of tobacco mosaic disease*

Temperature	Number of necroses	
	On the 4th day	On the 9th day
15° C.	16.5	58.6
20° C.	55.0	89.4

ethyl ether per litre as intensively as without ether. In higher concentrations of ether the accumulation of the virus slackens, but here is observed a certain injury of the leaves. In an atmosphere of hydrogen the tobacco leaves suffer too much to allow the solving of the question whether the cessation of the accumulation of the virus is due to the deprivation of the leaves of oxygen or is a by-product of the poor condition of the cells (11). Experiments of Woods (19) analogous in method, give evidence that the accumulation of the virus depends on an oxidative system, reversibly inactivated by CN.

The above examples show the ways where we sought answers to the questions concerning the biological activity of the virus. Certainly these investigations should be accompanied by direct observations of the cell affected by virus disease and cannot be separated from the structural conditions of autoreproduction of the virus. The paracrystalline and crystalline condition of the virus in the protoplasm must play an important role in the mechanism of its accumulation (10). It is possible that in the future the applications of Cassperson's (3) method to a cell affected by virus disease will render a significant service for our deeper understanding of the relation between the protoplasm and the virus, four our further understanding of the mechanism of its action and autoreproduction—questions which, we believe, occupy a central position in contemporary biology.

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A NEW SPECIES OF FUSARIUM CAUSING VASCULAR WILT OF TOMATO

FREDERICK L. WELLMAN[1]

(Accepted for publication March 29, 1943)

The purpose of this note is to remind plant pathologists that a new species of *Fusarium*, causing vascular wilt of tomato, has been known for some time,[2] and to so describe it that other investigators may watch for and recognize it. The original isolate came from V. A. Wright, formerly of

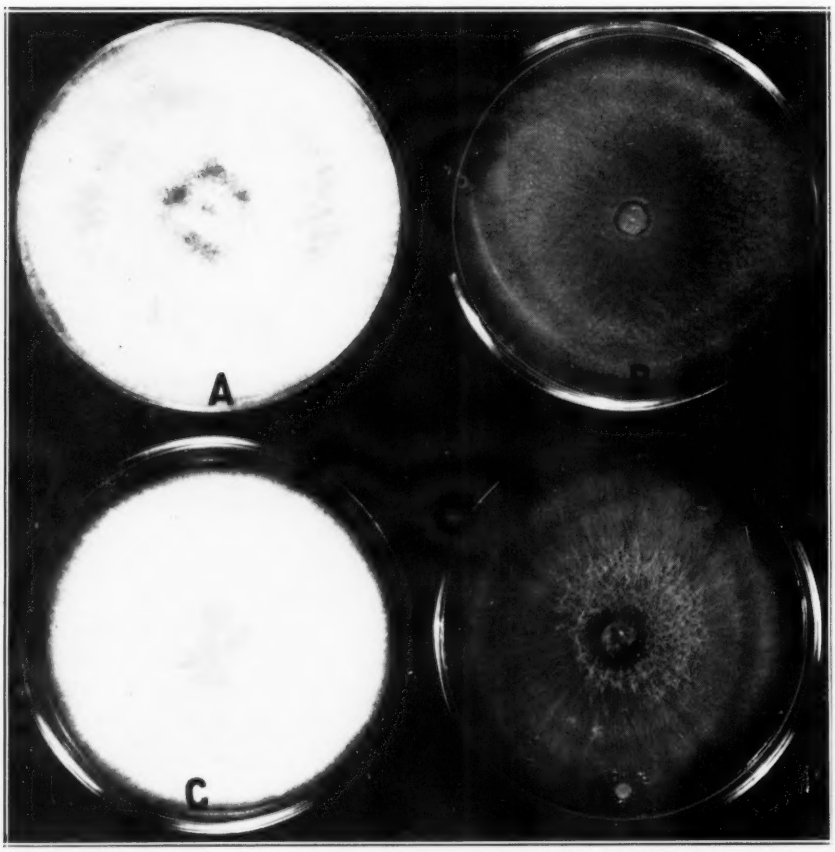

FIG. 1. Comparative growth in Petri-plate cultures of *Fusarium retusum* (A, raised type, note coarse aerial growth completely filling air space in dish; B, appressed type) and of *F. bulbigenum* var. *lycopersici* (C, raised type, fine, woolly; D, appressed type).

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[2] Wellman, F. L., and Dorothy J. Blaisdell. U. S. Dept. Agr. Techn. Bull. 705. 1940.

the Department of Botany of the Indiana Agricultural Experiment Station, with the notation: "Isolated November 1937, from a fruit borne by a plant showing severe wilting. The vascular tissue in the fruit itself showed a darkening in color. The plant was grown in a wilt-infested field near Trafalgar, Indiana." Dr. Wright released the material to me for taxonomic treatment.

Bonny Best and Marglobe tomato plants were inoculated with the Trafalgar organism. Wilt symptoms resulted, identical with those caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and Rank. Comparative cultural and microscopic studies were made of these two organisms. A search through all available literature dealing with *Fusarium* disclosed no described species to which the Trafalgar organism could be assigned. Morphologically, it was widely divergent from *F. bulbigenum* var. *lycopersici*, and was, therefore, unquestionably different from the new form of the latter reported by Reinking.[3] It was found by cultural manipulations that selected isolates of the new species could be separated into the same cultural classes as have been reported[2] for the common tomato wilt *Fusarium*.

TECHNICAL DESCRIPTION OF **Fusarium retusum** SP. NOV.

Growth on potato dextrose agar (Fig. 1) formed a slightly coarse, loose, and fluffy, rather "wild-growing" mat that, within a few days, filled the air space above the medium in the Petri dish or test tube. Colors[2] varied from white, livid-pink, and shades of yellow, through orange, tawny-olive, and garnet-brown, to Morocco red. A few sclerotia developed; at first white, becoming greenish. Cream-color sporodochia developed consistently in few monosporic isolations, but never in mass isolates. Reisolation and histological studies demonstrated infection hyphae growing only inside xylem elements of diseased tomato stems. With regard to morphology (Fig. 2) hyphae of fungus culture, hyaline, septate, branched, may be faintly tinged with yellow or red, and range from 2 μ to 7.5 μ in diameter; sporophores hyaline, bostrychoid, much branched, bearing easily detached microspores and macrospores; macroconidia, colorless and hyaline, straight to slightly curved, with a rounded, blunt apex and a distinct foot on the proximal end, 1–5-septate, mostly 3-septate, with average dimensions of about 26.8 μ long and about 4.3 μ wide; microconidia hyaline, colorless, mostly oblong and umbonate at the base, largely unicellular, rarely 1-septate, with average dimensions about 9.9 μ long and about 3.8 μ wide; chlamydospores may vary greatly in size and shape, are borne mostly in intercalary positions in mycelium or in macrospores, are colorless, and densely filled with protoplasmic granules. Natural occurrence in darkened vascular system in fruit of a severely wilted tomato plant near Trafalgar, Indiana.

Hyphae fertiles incoloratae, valde ramosae, bostrychoideae, et macroconidia et microconidia ferentes; macroconidiis hyalinis, incoloratis, rectis vel paulum curvatis, apice retusis, basi vulgo oblique pedicellatis, 1–5 septatis plerumque 3-septatis, saepius circa 26.8 μ longis et circa 4.3 μ crassus; microconidiis hyalinis, incoloratis, vulgo oblongatis et basi umbonatis, plerumque continuiis rarius uniseptatis, saepius circa 9.9 μ longis et circa 3.8 μ crassis. Chlamydosporae in mycelio etiam in macroconidiis ortae, plerumque intercalares, incoloratae, protoplasmatis granulosi repletae. Habitat in fructibus Lycopersici esculenti languidi prope Trafalgar, Indiana.

The well-known tomato organism, *Fusarium bulbigenum* var. *lycopersici*, is much slower in growth than *F. retusum*, and also varies from it in having much longer and more slender macrospores with quite pointed ends. My studies indicate that *F. retusum* is well outside the systematic limits of either the *Elegans* group[4,5] or the species *F. oxysporum*[6] with which the

[3] Reinking, O. A. Farm Research. New York (Geneva) 3: 12–13. 1937.
[4] Wollenweber, H. W. Fusaria Autographic Delineata. Berlin, 1930.

well-known organism is closely associated. It would appear from its cultural and morphological characters, that *F. retusum* may be considered a member of the systematic group *Sporotrichiella*[4,5] and possibly approaches nearest to the species *F. poae* (Pk.) Wr. It differs from this latter species,

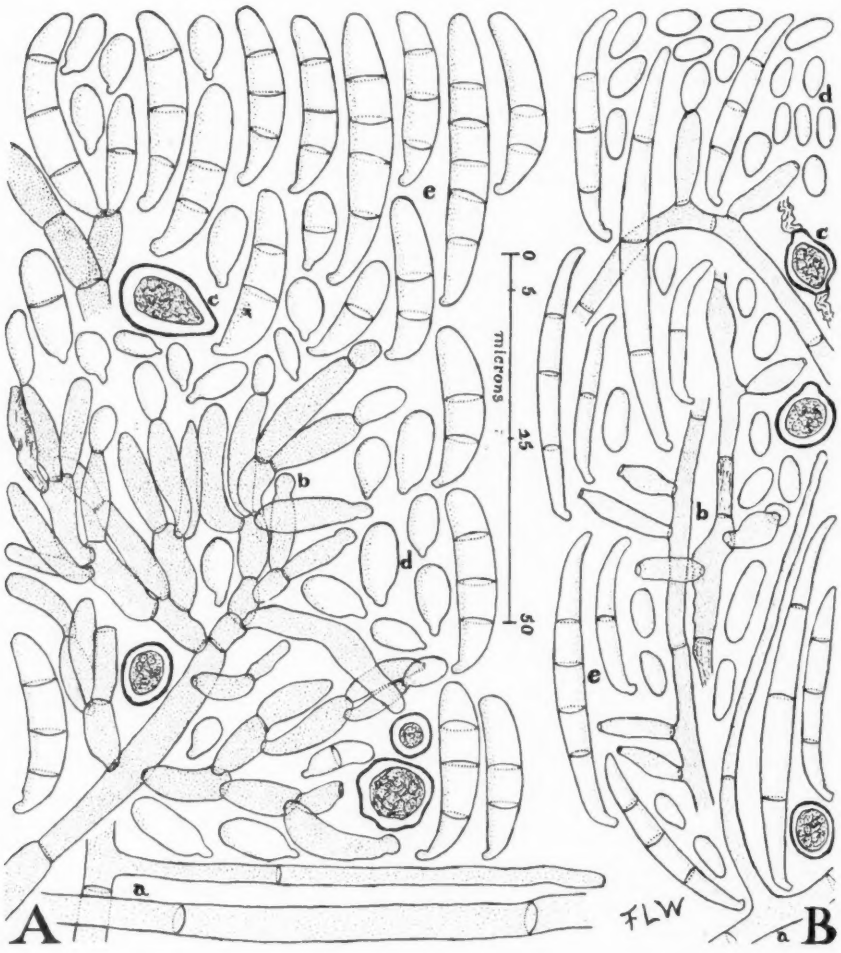

FIG. 2. Comparative morphology of (A) *Fusarium retusum* and (B) *F. bulbigenum* var. *lycopersici*. a, Mycelium; b, conidiophores; c, chlamydospores; d, microspores; e, macrospores. Magnification × 1000; drawings made with aid of camera lucida.

however, in the shape of its macrospores, whose blunt and rounded distal ends are very distinctive. The appellation *retusum*, which I have given to the new species, is from the Latin, meaning blunt. (I am indebted to Charles Drechsler for writing the Latin diagnosis.)

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[5] Wollenweber, H. W., and O. A. Reinking. Die Fusarien. Berlin, 1935.
[6] Snyder, W. C., and H. N. Hansen. Amer. Jour. Bot. 27: 64–67. 1940.

A BACTERIAL LEAF SPOT AND BLIGHT OF THE RUSSIAN DANDELION[1]

JOHN S. NIEDERHAUSER[2]

(Accepted for publication, July 27, 1943)

In the course of an investigation of diseases of the Russian dandelion, *Taraxacum kok-saghyz* Rod., a severe leaf spot and blight was noted. When first observed in early September, 1942, the disease was common to nearly 15 per cent of the plants in the test plot at Ithaca, New York, and it gradually became more severe until checked by a frost in mid-October. During this time there was much cloudy, wet weather.

The lesions occur on the leaves only, and vary in size from minute dots to large necrotic areas that may occupy nearly the entire blade. The diseased areas are uniformly black in color, with a narrow, yellow, chloranemic border. If there are large lesions near the base of the leaf, the distal portions may turn prematurely yellow. Ordinarily, the older, outer leaves are most severely affected.

Dilution plates made from bits of diseased leaf tissue constantly yielded a yellow bacterium that produced characteristic spreading, gummy, moist-shining colonies on potato-dextrose agar. It was immediately suspected that this organism was the cause of the disease. Inoculation experiments were carried out on Russian dandelion plants in the greenhouse with pure cultures of this bacterium, and lesions typical of those observed in the field were produced. Infection resulted when uninjured leaves were atomized with a water suspension of a young culture of the bacterium, and also occurred where the leaves were pricked with the point of a fine needle.

In the older leaves the lesions were extensive and spreading, while on young leaves they generally remained small and rarely exceeded 1 or 2 mm. in diameter. From these artificially induced lesions the same organism was consistently reisolated in pure culture.

In the field the roots have not been observed to be affected by the bacterium, and several attempts to induce infection of the roots through artificial inoculation were not successful.

Repeated inoculations on the common dandelion (*Taraxacum officinale* Weber), Cos lettuce (*Lactuca sativa* L. var. *longifolia* Lam.), head lettuce (*L. sativa* L. var. *capitata* L.), leaf lettuce (*L. sativa* L. var. *crispa* L.), and prickly lettuce (*L. scariola* L.) all failed to give infection.

Sigriansky (6) reported a "black necrosis" of the related Russian species, *Scorzonera tau-saghyz* Lipch. and Bosse, and gave the following description of the disease: "First to appear are small, black dots, which quickly coalesce to form much larger spots. The secretion of ooze drops has been

[1] Cost of publication borne by an outside agency.

[2] The writer wishes to express his appreciation of the indispensable help and advice given by Professor W. H. Burkholder during the progress of this work.

seen. The disease may be spread throughout the field, causing a premature dying of the leaves. The pathogen is apparently carried over in the seeds, and is spread with the help of insects, the wind, or soil particles, and especially in irrigating." This brief description suggests the same disease as that found at Ithaca on *Taraxacum kok-saghyz*, and also indicates that the pathogen may have been introduced with the seed imported from the Soviet Union. Sigriansky did not identify the pathogen except to infer indirectly that the causal agent is a bacterium.

In making the routine tests to describe the causal organism, 7 separate isolates were used, 1 of which descended from a single cell. All 7 isolates behaved similarly. In making the measurements the Congo red negative stain was employed. Plimmer and Paine's (5) modification of the Casares-Gil flagella stain was used to demonstrate the polar flagellum. The test for indol production in tryptophane broth was performed by the Ehrlich-Bohme technique (2). To determine the production of hydrogen sulphide from tryptophane broth, strips of filter paper impregnated with lead acetate were suspended over the cultures. The synthetic nitrate medium, described in the Manual of Methods (7), was used in the nitrate studies. Clara's dilute medium (3) was used to determine whether asparagin could be used as a combined carbon and nitrogen source. The lipase test was made according to the method outlined by Starr (8).

In making the biochemical tests to determine substances that can be used as a carbon and energy source, a synthetic basal medium, as given in the Manual of Methods (7), was prepared, with brom thymol blue added as an indicator. This basal medium was adjusted to pH 7, tubed, and autoclaved. The carbohydrates to be tested were prepared in 7.5 per cent aqueous solutions, sterilized by filtration, and then added aseptically to the tubes of basal medium, so that 0.75 per cent solutions were obtained. Sodium salts of the organic acids were added directly to the basal medium so as to make a 0.15 per cent solution, and autoclaved together. The test for the hydrolysis of starch was made by the starch-agar iodine method.

DESCRIPTION OF PATHOGEN

Morphology: The bacterium is a rod and occurs singly or in pairs. Individuals from a 24-hour culture on potato-dextrose agar at 27° C. give measurements of 1.4–3.3 $\mu \times .7$–1.2 μ (average 0.9 $\mu \times 2.3$ μ). It is motile with one polar flagellum, and is Gram-negative. No spores are formed.

Cultural Characters: On beef-extract peptone agar the colonies are circular, smooth, and bright yellow; growth is moderate. On potato-dextrose agar growth is abundant, pale-yellow, mucoid, and wet-shining. Beef-extract peptone broth becomes turbid, usually with a thin ring. In milk, growth is good and litmus is reduced; a soft casein curd is precipitated and then slowly digested; the supernatant liquid gradually clears and tyrosine crystals are abundantly produced. The optimum temperature for growth is about 30° C. Maximum and minimum temperatures for growth are 38° C. and 0° – 3° C., respectively.

Biochemical Characters: Growth in gelatin is good, and liquefaction occurs rapidly. No gas is produced in a beef-extract peptone broth plus 1 per cent dextrose. Growth in tryptophane broth is good; hydrogen sulphide is produced but not indol. Nitrate and ammonium salts can be utilized as a source of nitrogen; asparagin cannot be used as a collective carbon and nitrogen source. Nitrates are not reduced to nitrites. Lipase is produced. Acid is produced from xylose, dextrose,

galactose, levulose, lactose, sucrose, and glycerol; no growth occurs with arabinose, maltose, raffinose, inulin, mannitol, ethanol, and salicin. Salts of acetic, citric, lactic, malic, and succinic acids are utilized with an increase in pH. Salts of formic, tartaric, salicylic, and benzoic acids are not utilized. Starch is hydrolyzed. Sodium chloride tolerance is between 3.25 and 3.5 per cent.

To the writer's knowledge there is no report in the literature of a species of bacterium pathogenic to the Russian dandelion, *Taraxacum kok-saghyz* Rod., and the species described here is distinct in pathogenicity, as well as in several morphological and biochemical characters, from *Phytomonas lactucae-scariolae* Thornberry and Anderson (9) and from *P. lactucae* (Yamamoto) Burkholder as given by Bergey *et al.* (1)[3]. The bacterium is hence described here as new. Its characters listed above designate it as a species of *Xanthomonas* Dowson (4); and the name proposed is *Xanthomonas taraxaci* n. sp.

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[3] Original article is in Japanese.

PHYTOPATHOLOGY NOTE

Prevention of Three Peach Diseases by Ferric Dimethyldithiocarbamate Spray.—In 1942 ferric dimethyldithiocarbamate (Fermate) was tested as a spray for control of 3 peach diseases occurring in Sacramento Valley, California, peach orchards.

Two treatments, 27 and 13 days, respectively, before harvest (September 8) were given for brown rot of ripening fruit, caused by *Sclerotinia fructicola* (Wint.) Rehm. On the day harvest started the incidence of fruit infection in one orchard was: nonsprayed, 11 per cent; Fermate 1 lb. to 100 gal. plus 4 oz. of a wetting agent to 100 gal., 4 per cent. In a second orchard the incidence of fruit infection was: nonsprayed, 19 per cent; Fermate 1–100 plus 4 oz. of a wetting agent to 100 gal., 4 per cent; liquid lime-sulphur 0.75–100 plus 4 oz. of a wetting agent to 100 gal., 8 per cent. All percentages are averages of 4 replications.

Peach trees were sprayed October 16 with Fermate, liquid lime-sulphur, and Bordeaux mixture. Within 1 week after spraying, rust caused by *Tranzschelia pruni-spinosae* developed abundantly on leaves of unsprayed trees. To determine the control by the sprays, counts were made October 28. According to the results in column 2, table 1, Fermate prevented leaf infec-

TABLE 1.—*Comparative effectiveness of different spray materials in the control of rust (Tranzschelia pruni-spinosae) and blight (Coryneum beijerinckii) on peach*

Treatment	Average number of lesions of rust per leaf	Average number of lesions of blight per 100 twigs
Unsprayed	18	156
Fermate 1.5–100	3	5
Fermate 1.5–100 plus lime 1–100	1	12
Bordeaux mixture 10–10–100	9	23
Lime-sulphur 4–100	1	65
Lime-sulphur 6–100	1	14

tion during this period equally as well as lime-sulphur and considerably better than Bordeaux mixture. There was a suggestion that the addition of lime to the Fermate increased its effectiveness.

Within 1 month after application of the sprays in this orchard, blight or shot-hole, caused by *Coryneum beijerinckii*, developed to a moderate extent on twigs. The control obtained with the different sprays during this period is given in column 3 of the table. Fermate appeared to be significantly more effective against blight than lime-sulphur 4–100, and as effective as lime-sulphur 6–100 and Bordeaux mixture 10–10–100.

It should be noted, however, that in general practice of peach spraying one application of spray in the autumn is expected to protect the twigs against infection by rust and blight until the beginning of the next season. In such circumstances the spray deposit is subjected to weathering for a

period of several months, and, in consequence, weather resistance becomes an important factor in determining the effectiveness of the spray. In the present tests, which covered periods of 12 days in case of rust and 1 month in case of blight, weather resistance of the spray deposit was not so thoroughly tested.—E. E. WILSON and C. E. SCOTT, Division of Plant Pathology and Agricultural Extension Service, respectively, University of California, Berkeley, California.

Bacterial Infection and Decay of the Inner Wood of Winter-injured Young London Plane Trees.—The unusual occurrence of frost cracks in 5-year-old London plane trees (*Platanus acerifolia* Willd.) on bottom land in a Maryland nursery was called to the attention of the writer in 1934. Pruning wounds that were not healing properly were noticed the following summer on many of the trees. Trees thus affected presented a generally unhealthy appearance.

All apparently unhealthy trees were found on examination to have their 3 innermost annual rings discolored and water-soaked. This condition terminated abruptly about 5 inches aboveground. This water-soaked internal condition reached the surface of open pruning wounds; and a condition resembling slime flux was present. This apparently acted to prevent callusing. In addition, many of the open pruning wounds apparently had served as a point of entry to a rot fungus. This fungus had decayed the tissues of the inner 3 rings as much as 6 inches in each direction from the pruning wound. Often the rot fungus had entered at more than one point on the tree and the decay was almost continuous. A number of the trees died or broke off. Frost cracks occurred in half of the diseased trees. Every tree with frost cracks was found to be one with water-soaked inner wood.

From the decayed portion of the wood a hymenomycete was isolated. No fruiting bodies were found on any of the trees, but tissue cultures of the fungus were compared by R. W. Davidson of the Division of Forest Pathology with those in the wood-decay collection of that Division and found to be similar to known isolates of *Polyporus versicolor* L. ex Fr.

From the water-soaked, discolored inner wood, Gram-negative, $0.25 \times 0.5\ \mu$, rod-shape bacteria were consistently isolated. Preliminary inoculation tests indicated that these bacteria were capable of killing green shoots of newly rooted plane tree cuttings, when inoculated into a needle prick, or the entire cutting, if inoculated through a cut in the bark. What appeared to be similar bacteria were isolated from cuttings that failed to callus well. That the bacteria did not readily attack healthy tissue of well-established trees was indicated when examination of some of the survivors of the original group of trees several years later showed the water-soaked condition still limited almost entirely to the innermost 3 rings.

The most probable explanation of the observed phenomena is in the conditions of the preceding winters. The winter of 1931–2 was unpre-

cedentedly warm from November to February, inclusive. Many kinds of plants started growth prematurely. This was followed by cold weather in March. From the 10th to the 13th, inclusive, minima were between 15° and 18° F. at the College Park Weather Station, located on higher and presumably warmer ground, and on the 19th 11° F. was recorded. A considerable snowfall also occurred. Killing or injury of parenchyma in the wood is supposed to have resulted, followed by invasion of the bacterium and the decay fungus. The general limitation of the infections to wood above the snow line and to the rings formed before the 1932 freeze supports this view. Wetwood and slime flux are commonly associated with bacterial infection of the wood. The frost cracks, probably limited to the wetwood trees, occurred during the subzero weather of February, 1934, and are regarded as consequences of the water-soaked condition.—BOWEN S. CRANDALL, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Athens, Ga.

The votes regarding formation of a Potomac Division have been canvassed. The following are the results:

226—voting in favor of the formation of a Potomac Division

81—opposed to the formation of a Potomac Division

This vote indicates approval of the Society for the formation of a Potomac Division as outlined in the petition presented to the membership.

Special Committee:

Signed: W. G. STOVER, *Chairman*
C. W. ELLETT
D. M. MCLEAN